

**Proceedings of the 37<sup>th</sup> Annual Aquatic Toxicity  
Workshop: October 3 to 6, 2010, Toronto, Ontario**

**Comptes rendus du 37<sup>e</sup> atelier annuel sur la  
toxicologie aquatique: du 3 au 6 octobre 2010,  
Toronto, Ontario**

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## Preface/Preface

The 37<sup>th</sup> Annual Aquatic Toxicity Workshop was held at the Marriott Downtown Eatons Centre in Toronto Ontario, from October 3-6, 2010. The Workshop included three plenary presentations, 160 platform and 74 poster papers. Total attendance was 420.

This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

L' 37<sup>ième</sup> atelier annuel sur la toxicité a eu lieu au Marriott Downtown Eatons Centre au Toronto Ontario, Octobre 3-6, 2010. L'atelier a donné lieu à 3 communications lors de séances plénières, 160 exposés d'invités d'honneur et 74 communications par affichage. 420 personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministre des Pêches et Océans.

## **Editors' comments/Remarques des éditeurs**

This volume contains papers, abstracts or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Graham and Ms. Monica Lyons for their assistance in the preparation of these proceedings.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longueur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs en chef voudraient remercier Dr. Jill Graham et Mme Monica Lyons dans la préparation de ces comptes rendus.

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## Dr. Donald F. Alderdice, pioneer of Canadian aquatic toxicology

November 19, 1924 – March 19, 2010

JOHN B. SPRAGUE, Salt Spring Island, BC

Don Alderdice was the earliest Canadian to tackle aquatic toxicology in a thoroughly scientific way. The high quality of his work was unique.

### Scientific career

In 1951-52, Don was in the Maritimes doing field work on the aquatic effects of forest spraying with DDT. In 1953 he started his lifelong career at the Pacific Biological Station in Nanaimo, with the Fisheries Research Board of Canada. He started toxicity tests on insecticides which might be less harmful than DDT. Then he broadened his work, and in the late 1950s, published four toxicity papers as senior author (see References). Don's early work was of remarkably high quality. His first toxicity publication was in 1957, and characteristically, it was in a refereed scientific journal. It was titled *Some effects of kraft mill effluent on young Pacific salmon* (Alderdice and Brett, 1957). The level of science was in considerable contrast to most routine tests of the 1950s and 1960s, which tended towards large pickle jars, some effluent, a couple of fish, and coming back in the morning to inspect mortality. Indeed, there were no pickle jars in Don's lab when I first visited in 1964. Specially-designed insulated test-tanks were supplied with neatly arranged plumbing and power conduits (Figure 1). Everything was continuous-flow, not static. Toxicant was added by proportional diluters which went by the name "kinking tee-tube" —in other words a fail-safe arrangement to maintain constant concentrations. All of this was most advanced for the time and I was impressed.



Figure 1. Don Alderdice rebuilding his laboratory in 1964.

The sophistication of Don's early work is best shown by his Ph.D. studies started in the 1950s. Results were summarized as three-dimensional response surfaces showing the toxicity of pentachlorophenol as modified by temperature, salinity and oxygen (Alderdice 1963, see

Figure 2). Both the statistical design and the presentation of results are impressive, indeed most surprising for that time. One must bear in mind that in those days, graphs could not be created by entering numbers into a computer spreadsheet: data were first plotted by hand on a piece of paper, then the curves were also drawn by hand.

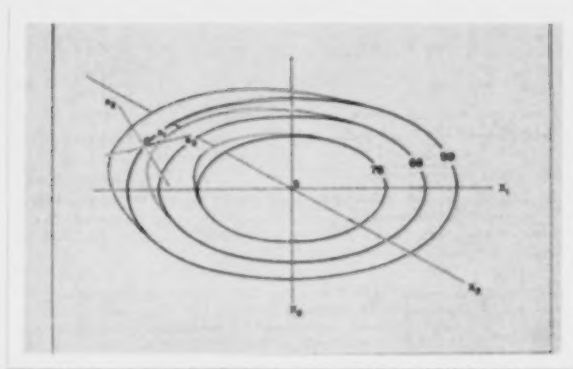


Figure 2.

Three-dimensional representation of the resistance of salmon to sodium pentachlorophenol, with varying

Don continued in this vein throughout his career, using his mathematical talents to support his multi-variate approach to environmental conditions acting on fishes. In later years, he focused on effects of low oxygen and other dissolved gases on eggs and developing young, work which he had also started in the 1950s (Alderdice *et al.* 1958, Alderdice and Wickett 1958). Before he retired in 1987, he worked on effects of supersaturation of gases, related to the effects of the many power-dams on British Columbia rivers. By then, Don probably considered himself a physiologist working on environmental stressors, but his findings are clearly of direct relevance to aquatic toxicology. Work on low oxygen is directly related to effects of organic pollutants.

#### Award

The quality of his work, and his multi-factorial approach, is confirmed by an award in 1974 from the Wildlife Society. This was for his 62-page chapter, *Factor combinations: responses of marine poikilotherms to environmental factors acting in concert*. The Wildlife Society pronounced it the "best publication in English on a fisheries subject, during the year." Quite a remarkable honour!

At the time of writing, Don's publication list has not been found. It has been partly recreated, and might be available for future workshop proceedings. Some idea of his recent titles may be seen at <http://scholar.google.com>.

#### The Person

Many tributes or memorials offer a picture of an old person but that is misleading. Don was a young man when he started his career, so some photographs from earlier years are shown below (Figure 3). We see a typical clean-cut graduate, an apparently happy and casual field worker perched precariously on the bow of a boat, and a guy who reportedly loved to jam at the piano. Clearly, aside from his persona as the careful and sober scientist, Don had other personality aspects.



Figure 3.

Early photos of Don Alderdice. Graduation photo, in a boat during field work, and at the piano for a Christmas party of the Pacific Biological Station. Photos supplied by Margot Herten, Don's daughter.



Don had a life-long marriage to Jean (nee Charters), who had deep family roots in New Brunswick. They had two daughters, Margot and Dawn Alison.

### An environmental crusade

At the time of his retirement in 1987, Don Alderdice became an environmental crusader. In the mid-1980s, there was a proposal to divert up to 78% of water from the

Nechako River, a major producer of sockeye salmon and the major tributary of the upper Fraser River. The idea was to drain it westward through a tunnel for power generation. (Earlier, Alcan at Kitimat B.C. had diverted approximately 30% of the river flow for that purpose, a level considered tolerable for the aquatic community.)

The new proposal had been assessed by a number of scientists at the Pacific Biological Station, and their opinion was unanimous that the bigger diversion would be a complete disaster for the river and its fish production. However, the government bureaucracy forbade the release of their reports and denied permission to discuss the matter in public.

The scientists chafed, but a few personalities from the news media were fighting a losing battle of opposition. Mysteriously, a plain brown envelope reached the news media with the scientific report. No one knows who sent it. Alderdice retired at that time and became a star witness at the hearings. He was joined by Dr. Gordon Hartman, another retired fisheries biologist at the Biological Station. Figure 4 shows these two in 1993 when the struggle was still going on. They devoted a great deal of time and effort to the hearings, and were widely acclaimed as heroes by the news media and First Nations.



Figure 4.

Dr. D.F. Alderdice (left) and Dr. Gordon Hartman in 1993, retired and campaigning environmentally in B.C. From an article in *Harrowsmith*, December 1993.

Although the diversion of water was initially granted, it appeared that the crusaders had won when the NDP provincial government of 1995 killed the proposal. Unfortunately, that decision was reversed a couple of years later.

A final photograph (Figure 5) shows a retired and apparently happy Don Alderdice in the late 1990s, on his balcony overlooking the sea in Nanaimo. With him is a colleague, Harald Rosenthal from Germany. Don worked with Harald during a highly productive two-year stint in Germany.

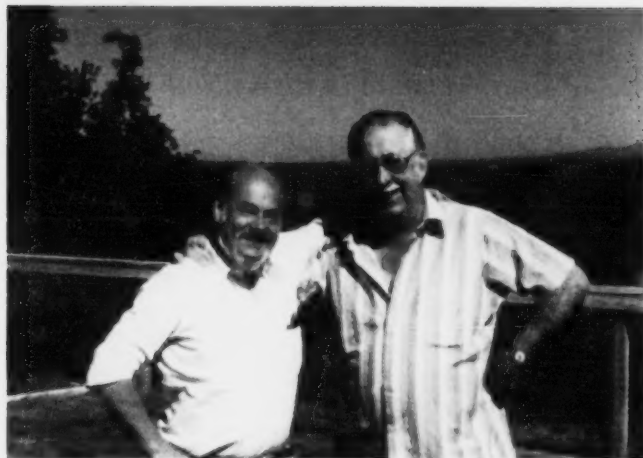


Figure 5.

Don Alderdice (right) in retirement, with colleague Harald Rosenthal at Don's residence in Nanaimo. Photo courtesy of Margot Herten.

The Don Alderdice Memorial Scholarship Fund for aquatic biologists has been set up at Vancouver Island University, Nanaimo (contact [foundation@viu.ca](mailto:foundation@viu.ca) for information). Don made a major donation to set up the F.E.J. Fry Scholarship for a biology student at the University of Toronto.

#### Early publications

- Alderdice, D.F. and J.R. Brett. 1957. Some effects of kraft mill effluent on young Pacific salmon. *J. Fish. Res. Board Can.* 14: 783-795.
- Alderdice, D.F. and J.R. Brett. 1957. Toxicity of sodium arsenite to young chum salmon. *Fish. Res. Board Can., Prog. Rept. Pacific Coast Stns* 108: 27-29.
- Alderdice, D.F., W.P. Wickett and J.R. Brett. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. *J. Fish. Res. Board Can.* 15: 229-249.
- Alderdice, D.F. and W.P. Wickett. 1958. A note on the response of developing chum salmon eggs to free carbon dioxide in solution. *J. Fish. Res. Board Can.* 15: 797-799.
- Brett, J.R. and D.F. Alderdice. 1958. The resistance of cultured young chum and sockeye salmon to temperature below 0 deg. C. *J. Fish. Res. Board Can.* 15: 805-813.
- Alderdice, D.F. and M.E. Worthington. 1959. Toxicity of a DDT forest spray to young salmon. *Can. Fish. Culturist* 24: 41-48.
- Alderdice, D.F. 1963. Some effects of simultaneous variation in salinity, temperature and dissolved oxygen on the resistance of young Coho salmon to a toxic substance. *J. Fish. Res. Board Can.* 20: 525-550.

#### Award winner

- Alderdice, D.F. 1972. Factor combinations: responses of marine poikilotherms to environmental factors acting in concert. In O. Kinne (ed.) *Marine ecology, vol. 1, Environmental factors, part 3*, p. 1659-1722. Wiley-Interscience, London.



## Winners of Dr. Richard Playle Award

PLAYLE AWARD FOR MSc: CAROLYN BROWN, University of Waterloo, Dept of Biology

### **Fish communities near municipal wastewater discharges in the Grand River Watershed**

Municipal wastewater effluent (MWWE) has the potential for aquatic degradation, as it is the largest, per volume, anthropogenic discharge in Canada and other areas in the world. With population increasing in many areas there is concern that infrastructure of wastewater treatment facilities will not be able to maintain adequate treatment and prevent further degradation of the environment. The Grand River watershed in Southern Ontario is predicted to have its population increase to 1.2 million people by 2031 (from 780,000 people in 2001). Although wastewater treatment has improved, concern remains for receiving environments due to inadequate treatment (e.g., Kitchener) and minimal dilution (e.g., Guelph). This research was conducted in order to understand current impacts on fish communities of MWWE in the Grand River watershed and to support future management and protection. Study sites upstream and downstream were chosen for their proximity to the Guelph, Kitchener, and Waterloo MWWE outfalls, similarity of habitat, and wadeability.

Habitat analysis indicated that there were no large physical differences among sites. Fish communities were collected in a standardized method with a backpack electroshocker at each site (six randomly selected 10 m by 10 m sub-sites for 5 min). Greenside darter (*Etheostoma blennioides*) and Rainbow darter (*E. caeruleum*), the most abundant species, were also analyzed for stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) at each site. Downstream of the Guelph outfall there were no changes in mean total catch per unit effort (CPUE) or mean total mass, but there was a decrease in Greenside darter abundance and an increase in Rainbow darter abundance. Downstream of the Kitchener discharge, there was a trend towards decreasing mean total CPUE, especially for darter species, and an increase in mean total mass due to a community shift to larger species including Catostomids and Centrarchids. Lower diversity downstream of all three MWWE outfalls can be attributed to the increase in Rainbow darter abundance. Stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of Greenside darter did not change downstream of the Guelph and Waterloo discharges, but signatures of Rainbow darter increased immediately below the two outfalls. This shift may be due to the Rainbow darter's ability to take advantage of a change in the environment (i.e. food availability), resulting in its increased abundance and changes in isotopic signature. Directly downstream of the Kitchener outfall both darter species had an increase in  $\delta^{13}\text{C}$  and a large decrease in  $\delta^{15}\text{N}$ , likely due to high nutrient inputs from the outfall. MWWEs are currently affecting the aquatic environment, including fish communities in the Grand River watershed. Future investments in infrastructure and watershed management should be made to mitigate degradation of water quality in this watershed.

**Assessment of exposure to sewage effluent and potential reproductive effects using the threespine stickleback (*Gasterosteus aculeatus*) as a monitoring species**

Previous research has shown that fish exposed to industrial or municipal effluents such as pulp and paper or sewage can exhibit reproductive dysfunction. These conditions can lead to a decrease in sex steroid hormone levels and changes in gonadal morphology. The threespine stickleback (*Gasterosteus aculeatus*) has become an ideal biomonitoring organism due to its completely sequenced genome and established biochemical indicators to simultaneously measure exposure to androgens and estrogens. It was hypothesized that *G. aculeatus* populations exposed to sewage effluent and food processing effluents would show qualitative and quantitative signs of exposure to exogenous androgens and estrogens or effects on reproductive biochemistry and physiology. The primary objectives of this study were to measure exposure to androgens and estrogens using transcript biomarkers (i.e. spiggin and vitellogenin), measure gonad sex steroid hormone levels, and determine gonad tissue level changes in threespine stickleback exposed to treated sewage effluent from two wastewater treatment plants on Prince Edward Island (Kensington and Cornwall). Sticklebacks were sampled in fall 2009 and spring 2010. An induction of vitellogenin was seen in males at the Cornwall site (Hyde Creek). Stimulated ovarian tissue in vitro produced significantly higher sex steroid hormones at the Kensington site (Barbara Weit Estuary). The aforementioned results, along with gonadal histopathology analysis, will also be compared to similar data collected from the 2010 sampling. Potential future studies could involve water analysis to detect possible androgens and estrogens present.

## Presentations

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### *Chemicals of Concern*

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#### **Ecotoxicity of a brominated flame retardant (tetrabromobisphenol A bis) and its derivatives in water and sediments**

*DEBENEST, T. <sup>1</sup>, GAGNÉ, F. <sup>1</sup>, KOHLI, M. <sup>1</sup> and BLAISE, C. <sup>1</sup>. <sup>1</sup>Environnement Canada/Environment Canada*

A large research program included in the Chemicals Management Plan of Environment Canada was launched to assess the toxic effects of a priority chemical, the tetrabromobisphenol A bis (B<sub>4</sub>BPA bis), which is widely used as a flame retardant (FR) in manufactures and industries although its toxicity, its behaviour and its biotransformation in the ecosystems are unknown or unpredictable. Due to the increased development of electric and electronic equipment containing TBBPA bis, this chemical represents a major threat for aquatic ecosystems. Nevertheless, the risk for aquatic biota of this widely used FR remained relatively unknown. In this context, the toxicity of this FR and its derivatives spiked in water and in artificial sediments with different granularities (Sand/Kaolin) was assessed using a battery of 7 bioassays with organisms representative of the different trophic levels in the aquatic ecosystems. The solid phase and elutriates of sediment were studied. The results highlighted no toxic risk for water and sediments contaminated by the B<sub>4</sub>BPA bis and the B<sub>1</sub>BPA. Otherwise, water sediments spiked with B<sub>2</sub>BPA, B<sub>3</sub>BPA and BPA presented the highest toxic risk. Adsorption of these compounds on the fine particles (Kaolin) is suspected, with toxicity of elutriates increasing in sandy sediments. These data will be fed into an artificial neural network to develop a SED-TOX model to assess the toxic risk of sediments.

#### **Oxy-PAH: Structure and toxicity**

*FALLAHTAFTI, S. <sup>1</sup>, RATANEN, T. <sup>1</sup>, HODSON, P. <sup>1</sup>, BROWN, S. <sup>1</sup> and SNIECKUS, V. <sup>1</sup>. <sup>1</sup>Queen's University*

Polycyclic Aromatic hydrocarbons (PAHs) are lipophilic environmental contaminants with petrogenic, biogenic, and pyrogenic sources. Alkyl-PAHs predominate in crude oils and are one of the main target analytes in natural resource damage assessments for oil spills. Studies suggest that some alkyl-PAHs such as retene (7-isopropyl-1-methylphenanthrene) are more toxic than their un-alkylated parent PAH, and may exhibit altered biological activity. Previous work in our lab points to a link between the enzymatic metabolism of alkyl-PAHs such as alkyl phenanthrenes (APs), the resulting generation of oxy-PAH metabolites in the form of ring (phenols) and chain hydroxylated (benzylic alcohols) derivatives, and the increased prevalence of toxicity in early life stages (ELS) of fish. It remains unclear whether this metabolic toxicity enhancement is attributed to the by-products of metabolism such as reactive oxygen species (ROS) and reactive intermediates, or the metabolites themselves. While the introduction of oxygen increases the polarity of the compound as a first step in



excretion, early work suggests these derivatives to be more toxic than their un-substituted counterparts. Typically, monitoring schemes used in the estimation of biological effects, ecological risk, environmental damage, and bio-remediation standards do not consider alkyl or hydroxyl derivatives and may substantially underestimate toxicity. The main objective of this research is to estimate the potential role of these oxy-alkyl-PAH derivatives in PAH metabolism and toxicity through assessment of the chronic toxicity of a series of ring and chain hydroxylated AP derivatives to the ELS of fish.

#### **Toxicity of four sulfonamide antibiotics to the freshwater amphipod *Hyalella azteca***

BARTLETT, A. <sup>1</sup>, BROWN, L. <sup>1</sup>, TOITO, J. <sup>1</sup> and BALAKRISHNAN, V. <sup>1</sup>. <sup>1</sup>Environment Canada

Sulfonamide antibiotics are widely used compounds; however, there is a lack of data available to conduct risk assessments of their environmental impacts. The goal of this study was to assess the aquatic toxicity of four sulfonamide antibiotics to *Hyalella azteca*, an invertebrate species that is ubiquitous in North American freshwaters. Juvenile *Hyalella* were exposed for four weeks in static-renewal, water-only systems to nominal concentrations ranging from 30-4000  $\mu\text{g L}^{-1}$  for sulfaguanidine, 80-10,000  $\mu\text{g L}^{-1}$  for sulfathiazole and sulfamerazine, and 40-5000  $\mu\text{g L}^{-1}$  for sulfasalazine. Survival was evaluated weekly, and both survival and growth were measured at the end of the tests. Relative sulfonamide toxicity, from most to least toxic, was: sulfaguanidine > sulfathiazole > sulfamerazine > sulfasalazine. Four-week LC50s for sulfaguanidine and sulfathiazole were 240  $\mu\text{g L}^{-1}$  and 660  $\mu\text{g L}^{-1}$ , respectively. Sulfamerazine data were variable, with LC50s occurring over a 10-fold range (440-3900  $\mu\text{g L}^{-1}$ ). Sulfasalazine showed no effects on survival or growth at any concentration tested. With the exception of sulfasalazine, sulfonamide toxicity increased with exposure duration. Growth was affected by sulfathiazole but was less sensitive than survival. Estimates of toxicity are currently based on nominal concentrations; it is likely that effect concentrations will decrease once measured concentrations are available. The effects observed in this study occur at levels well above those typically found in municipal wastewater effluent, but within the range reported for localized areas receiving agricultural run-off.

#### **Does salinity affect the response to 17 $\alpha$ -ethynylestradiol in the estuarine mummichog (*Fundulus heteroclitus*)?**

CHANDRA, K. <sup>1</sup>, NADON, T. <sup>2</sup>, LISTER, A. <sup>2</sup>, MACLATCHY, D. <sup>2</sup> and CURRIE, S. <sup>1</sup>. <sup>1</sup>Mount Allison University, <sup>2</sup>Wilfrid Laurier University

17 $\alpha$ -ethynylestradiol (EE2) is an endocrine disrupting chemical (EDC) that has deleterious effects in fish. Vitellogenin (Vtg), the fish egg yolk precursor protein, is an ideal biomarker of EE2 susceptibility. Most studies investigate endocrine-disrupting effects under stable fresh water conditions but it is unclear how fish respond to EDCs in changing salinities. Since the mummichog can move between fresh and salt water, the goal of our study was to determine if susceptibility to EE2 is affected by fresh or salt water. Due to the importance of heat shock proteins (HSPs) in the stress response and in estrogen signaling, we also determined if EE2 exposure affected the induction and cellular localization of HSP70 and HSP90. We exposed male mummichog, acclimated to either fresh or brackish (16 ppt) water, to 250 ng L<sup>-1</sup> EE2 for 7 days. Quantitative PCR was used to determine the hepatic mRNA expression of Vtg1 and Vtg2. Furthermore, liver samples were separated into nuclear,

cytoplasmic and membrane fractions, and immunoblotted for HSP70 and HSP90. As expected, Vtg expression was significantly higher in fish exposed to EE2. Interestingly, this Vtg induction was approximately 10 times greater in fish exposed in brackish water, indicating increased susceptibility to EE2. On the other hand, HSP70 and HSP90 did not change in any of the fractions across any treatments. Our results to date demonstrate that the physical environment plays a significant role in endocrine disruption, but the cellular mechanism by which this occurs is still unclear.

### **An aquatic toxicological evaluation of sulphate: The case for considering hardness as a modifying factor in setting water quality benchmarks**

*GILRON, G.<sup>1</sup>, ELPHICK, J.<sup>2</sup>, DAVIES, M.<sup>3</sup>, CANARIA, E.<sup>2</sup>, LO, B.<sup>2</sup> and BAILEY, H.<sup>1</sup> Teck Resources Limited, <sup>2</sup>Nautilus Environmental, <sup>3</sup>Hatfield Consultants*

Elevated concentrations of sulphate occur commonly in anthropogenically-impacted and natural waters. However, water quality guidelines have not been developed in many jurisdictions, and chronic toxicity data are scarce for this anion. A variety of test organisms, including species of invertebrate, fish, algae, moss and an amphibian, were tested for chronic toxicity in order to develop a robust dataset which could be used to develop water quality benchmarks. Guidelines were calculated using two standard procedures: 1) a species sensitivity distribution (SSD) approach, following methods employed in developing Canadian water quality guidelines; and 2) a safety factor approach, according to procedures typically used in the development of provincial water quality guidelines in British Columbia. The interaction of sulphate toxicity and water hardness was evaluated and incorporated into the guideline calculation, resulting in proposed guidelines for: soft (i.e., 10 to 40 mg L<sup>-1</sup>), moderately hard (i.e., 80 to 100 mg L<sup>-1</sup>) and hard water (i.e., 160 to 250 mg L<sup>-1</sup>). The resulting guideline values were: 129, 644 and 725 mg L<sup>-1</sup> sulphate, respectively, following the SSD approach, and 75, 625 and 675 mg L<sup>-1</sup> sulphate, following the safety factor approach.

### **Challenges associated with evaluating substances of emerging concern in the Great Lakes, based on monitoring and effects data**

*SCHROEDER, J.<sup>1</sup>, HULL, R.<sup>2</sup>, KLEYWEGT, S.<sup>1</sup>, FEISTHAUER, N.<sup>1</sup>, TEED, S.<sup>2</sup>, JESUPILLAI, A.<sup>1</sup> and FLETCHER, T.<sup>1</sup> Ontario Ministry of the Environment, <sup>2</sup>Intrinsic Environmental Sciences Inc.*

In 2009, the Ontario Ministry of the Environment and Intrinsic Environmental Sciences Inc. initiated a project under the Canada-Ontario Agreement Respecting the Great Lakes Basin Ecosystem (COA) – Annex 2 (Harmful Pollutants) to screen substances of emerging concern in the Great Lakes. The objective of the screening project was to determine how substances might be prioritized for further programming consideration under COA – Annex 2. Monitoring data from various Great Lakes programs were reviewed, courtesy of the International Joint Commission's Work Group on Chemicals of Emerging Concern, which compiled data on over 300 chemicals in the Great Lakes Basin water, sediment, and biota. Four criteria were applied to focus the initial list of substances to those that could be most clearly linked to Canadian sources and with a potential concern. Where available, existing environmental criteria were used to compare to concentrations measured in the environment and, where no environmental criteria existed, toxicity-based benchmarks were developed. Generally, the results of the screening assessment showed that concentrations of substances are below

those associated with conventional toxicological effects such as mortality, growth and reproduction, but conventional risk-screening approaches that employ monitoring data and traditional toxicity endpoints may not be sufficient to evaluate the potential for subtle long-term effects of substances of emerging concern in the Great Lakes. The approach and results of the screening exercise will be discussed along with limitations of the screening criteria, monitoring and toxicological data.

### **National survey of disinfection by-products and selected emerging contaminants in Canadian drinking water: Monitoring of pharmaceutical compounds (PO)**

BRAGG, L. <sup>1</sup>, TUGULEA, A. <sup>2</sup> and SERVOS, M. <sup>1</sup>. <sup>1</sup>University of Waterloo, <sup>2</sup>Health Canada

An area of growing interest for water research is the occurrence of many emerging contaminants in drinking water, particularly pharmaceuticals. This study is a part of a larger drinking water surveillance program, looking at various disinfection by-products, inorganics and emerging contaminants across Canadian provinces and territories. Thirty five drinking water plants, with various water treatment processes, water sources and populations, were selected for the first year of the two years study. Sites were visited during summer and winter sampling campaigns of 2009. At each site, duplicate samples were taken from the raw water prior to treatment, and from the final treated drinking water. Samples were spiked onsite with surrogate standards for all pharmaceuticals of interest (with the exception of lincomycin and monensin, where isotopically labelled standards were unavailable) and preserved. Sample extraction was performed at the University of Waterloo, using solid phase extraction with Oasis HLB cartridges. The extracts were analyzed for 21 selected analytes on an Agilent 1200 liquid chromatograph using an Applied Biosystems MDS Sciex 3200 Qtrap mass spectrometer detector with electrospray ionization. Both positive and negative ionization were used to quantitatively determine a wide range of analytes. Concentration and occurrence data will be presented for both summer and winter sampling campaigns. For the majority of analytes, occurrence was increased in the summer compared to the winter sampling. Overall, concentrations of all pharmaceuticals and emerging contaminants were found to be very low or below the detection limits in both sampling periods. Some treatment plants were able to successfully remove these contaminants through their treatment process, whereas some plants were less efficient in this respect. The analytes with highest percent occurrence were atrazine, caffeine, carbamazepine, diclofenac, ibuprofen, naproxen and venlafaxine.

### **Perfluorinated compounds in snapping turtle plasma from two Canadian Areas of Concern (PO)**

DE Solla, S. <sup>1</sup>, PELLETIER, E. <sup>1</sup> and LETCHER, R. <sup>1</sup>. <sup>1</sup>Environment Canada

Perfluorinated compounds (PFCs) are a broad class of chemicals that are used as stain repellents, surfactants, flame retardants, and in other applications, but also include degradation products of other PFC precursors. Seventeen PFCs were measured in plasma of male snapping turtles (*Chelydra serpentina*) collected from 3 sites in Ontario, including two Areas of Concern; the reference site Lake Niapenco (Binbrook), Humber River (Toronto AOC), and Cootes Paradise (Hamilton Harbour AOC). Both AOC sites were within large urban areas, and both were close to sewage treatment plants (STPs). Lake Niapenco is a rural site, with no

nearby STPs or local industry. Unexpectedly, mean concentrations were highest at Lake Niapenco ( $2.1 \mu\text{g g}^{-1}$ ), compared to the Humber River ( $0.12 \mu\text{g g}^{-1}$ ) and Cootes Paradise ( $0.53 \mu\text{g g}^{-1}$ ). PFOS and PFHxS comprised ~99.8% of sum PFCs at Lake Niapenco, and 86-88% at the other two sites. PFOS and PFHxS were both used in fire fighting foams. There is an important but currently unidentified source of PFCs, particularly PFOS, in or upstream of the rural "reference" site. Future work may be focused on attempting to identify this source.

### **Sublethal toxicity of CMP dyes to fish (PO)**

PARROTT, J. <sup>1</sup> and BALAKRISHNAN, V. <sup>1</sup>. <sup>1</sup>Environment Canada

We assessed several anthraquinone and anthracene dyes from Canada's Chemicals Management Plan (CMP) to determine sublethal toxicity in the fathead minnow. Data show toxicity to fish at low concentrations ( $32 \mu\text{g L}^{-1}$  nominal, approx  $10\text{-}15 \mu\text{g L}^{-1}$  measured) of Disperse Yellow 7. Sudan Red was also toxic to fathead minnow larvae at  $100 \mu\text{g L}^{-1}$ . Acid Blue 80 was non-toxic at levels of  $10 \text{ mg L}^{-1}$ , nominal concentrations. Based on previous data, we expect the measured concentrations to be about one-third to one-half of the nominal concentrations. Data from fathead minnow showed a delayed toxicity response, with larval fish succumbing 4-5 days after hatch. This is important for risk assessment fish bioassay data that may assess fish toxicity in embryo or at hatch, where toxicity of these compounds would be greatly underestimated. These data will be used in CMP risk assessments, and will be compared to expected or measured environmental concentrations, in order to determine if these dyes pose a potential concern to Canadian environments downstream of textile dyeing facilities or municipal wastewater outfalls.

### **Cellular vacuolization is induced in fish cells by environmental pharmaceuticals of the amine drug class (PO)**

WONG, J. <sup>1</sup>, BROMAND, S. <sup>2</sup>, SAFFI, G. <sup>1</sup>, BLOCH, S. <sup>1</sup>, LEE, L. <sup>3</sup>, HODSON, P. <sup>4</sup> and BOLS, N. <sup>1</sup>.

<sup>1</sup>University of Waterloo, <sup>2</sup>University of Toronto, <sup>3</sup>Wilfrid Laurier University, <sup>4</sup>Queen's University

Vacuolization of cells is an example of an easily observed indicator of a disruption in cellular homeostasis and was explored in eel and rainbow trout cell cultures as a marker and as a mechanism of action for environmental pharmaceuticals. Early passage cell cultures from the American eel (*Anguilla rostrata*) were prepared and studied because eel populations have been declining dramatically since 1975 in the St Lawrence River basin. Rainbow trout cell lines also were used because they are well characterized and have been widely used in environmental toxicology. The pharmaceuticals examined were basic amine drugs such as procaine, ranitidine, procainamide, propranolol, which end up in the environment as a result of their high usage as anaesthetics and stomach and heart medications. Without changing cellular metabolism or plasma membrane integrity, high doses of these drugs caused massive vacuolization in cells within 3-4 hours of exposure and this could be quantified by an increase in neutral red uptake. Vacuolization was particularly prominent in gill epithelial cells, suggesting how these drugs might have chronic and acute effects on fish.



## **Role of biota in the transport and fate of PCBs in remote areas: Salmonids in Patagonia and penguins in Antarctica (PO)**

BAHAMONDE, P. <sup>1</sup>, BARRA, R. <sup>2</sup>, RUDOLPH, I. <sup>2</sup>, MENDOZA, G. <sup>3</sup> and QUIROZ, R. <sup>4</sup>. <sup>1</sup>Canadian Rivers Institute, <sup>2</sup>EULA Environmental Sciences Centre, <sup>3</sup>Fisheries Research Institute, <sup>4</sup>Patagonian Ecosystem Research Center

The Chilean Patagonia and Antarctic are two of the few places worldwide considered pristine areas, but they have been subjected to anthropogenic impacts that ended in contamination by PCBs. Two cases are reported. PCBs were analyzed in salmonid muscle tissue from Patagonia and penguin feces in the Antarctic Peninsula by gas chromatography equipped with electron capture detector (GC-ECD). PCBs were found in three salmonid species, chinook salmon had the higher concentrations with an average of 5.74 ng g<sup>-1</sup> wet wt., followed by coho salmon and fario trout with 3.67 and 1.25 ng g<sup>-1</sup> wet wt. respectively. In penguin species, the highest levels were found in Gentoo Penguin with an average of 28.0 ng g<sup>-1</sup> wet wt. against Chinstrap & Adelia Penguin with 5.1 y 10.3 ng g<sup>-1</sup> wet wt. respectively. Migrating salmonids presented the highest observed PCB levels in their tissues; however the PCB patterns were rather similar in all species, suggesting a common source (i.e. food). In the penguin's case, notable differences were observed in the tested species, these differences could be explained by variation in dietary food. This project was supported by FONDECYT 1080294 and INACH T-18-09, and the Army's Antarctic Department is also acknowledged.

## **In vivo detection of emerging contaminants in fish using solid phase micro-extraction (PO)**

WANG, S. <sup>1</sup>, OAKES, K. <sup>1</sup>, BRAGG, L. <sup>1</sup>, PAWLISZYN, J. <sup>1</sup>, DIXON, D. G. <sup>1</sup> and SERVOS, M. <sup>1</sup>.  
<sup>1</sup>University of Waterloo

Emerging contaminants of concern, including pharmaceuticals and personal care products (PPCPs), can reach surface waters, ground waters, and sediments through the release of sewage effluents into receiving environments thereby posing a potential threat to aquatic organisms. The feasibility of *in vivo* determination of contaminants in live fish to avoid lethal sampling using solid phase micro-extraction (SPME) has been validated by this study and SPME was successfully applied to quantitate contaminants in sewage effluents as well as in exposed fish to track the bioaccumulation process. SPME was contrast against conventional extraction techniques including solid phase extraction (SPE) and solid liquid extraction (SLE) to assess the accuracy of SPME. These techniques were compared to quantify eight selected emerging contaminants (atrazine, carbamazepine, naproxen, diclofenac, gemfibrozil, bisphenol a, fluoxetine and ibuprofen) in controlled chemical mixture exposures in the laboratory as well as in field sampling of wild fish collected from sites near effluent outfall. Results indicated six target compounds including diclofenac, naproxen, gemfibrozil, ibuprofen, bisphenol a, and carbamazepine were found in the sewage effluents and they were also identified *in vivo* in exposed fish. SPME was concluded as an excellent alternative extraction technique for quantitative determination of contaminants in sewage effluents and it has the advantage over conventional techniques due to its suitability for *in vivo* monitoring of bio-accumulated contaminants in tissues of living organisms.

## **Determination of Cyclic Volatile Methyl Silicones (cVMSs) in influent and effluents of municipal water treatment plants (PO)**

STEER, H. <sup>1</sup>, YOUNG, T. <sup>1</sup>, TAIT, T. <sup>1</sup>, WANG, D. <sup>1</sup>, PACEPAVICIUS, G. <sup>1</sup>, NG, T. <sup>1</sup>, SMYTH, S. <sup>1</sup>, KINSMAN, L. <sup>1</sup> and ALAEE, M. <sup>1</sup>. <sup>1</sup>Environment Canada

Cyclic volatile methyl silicones (cVMSs) are high volume chemicals that are used in a wide variety of industrial applications and personal care products. In 2009, the Government of Canada concluded that the cVMSs Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5) present a potential harm to the aquatic environment and declared them as CEPA toxic compounds. As a result, the determination of the sources and fate of these pollutants in water is of interest to Environment Canada. cVMSs are released into the aquatic environment through industrial activities and the use of personal care products and, ultimately end up in the municipal water treatment plant. Physical and chemical properties of these compounds such as high volatility, low water solubility and high lipophilicity indicate that these compounds prefer to partition to air and biosolids. This represents a wide analyte concentration range and may lead to challenges in sample analysis. Therefore a reliable, versatile and robust method for determination of these compounds was required.

In order to meet the requirements for these measurements an isotope dilution method based on membrane enhanced solvent extraction (MASE) combined with large volume injection (LVI) GC/MS was developed. Influent and effluent samples from several municipal water treatment plants from Southern Ontario and Quebec were collected and analyzed using this method. Method validation and results obtained from these samples are discussed in this presentation.

## **DNA mutation rate in double-crested cormorants (*Phalacrocorax auritus*) associated with exposure to PAHs on Lakes Ontario and Erie (PO)**

KING, L. <sup>1</sup>, DE SOLLA, S. <sup>2</sup> and QUINN, J. <sup>1</sup>. <sup>1</sup>McMaster University, <sup>2</sup>Environment Canada

Pollution from steel production is a threat to nearby wildlife and a serious concern near Hamilton, Ontario, home to two integrated steel mills. Research suggests this air pollution causes higher DNA mutation rates in herring gulls and mice, with Polycyclic Aromatic Hydrocarbons (PAHs) as a suspected cause. Cormorants are an excellent species in which to study airborne and dietary exposure as they eat almost entirely fish, simplifying the tracing of the aquatic route of contaminant exposure. We collected blood and regurgitated samples from Double-crested Cormorant (*Phalacrocorax auritus*) families in two colonies adjacent to steel mills in Hamilton Harbour (Lake Ontario) and one colony at our reference site on Mohawk Island (Lake Erie), Ontario. DNA from the blood samples is being used to determine the germline mutation rate in each colony using several microsatellite loci. To understand dietary exposure, we will investigate whether adult cormorants are foraging from littoral or pelagic food webs, and whether varying diets can cause differing contaminant uptake. Red blood cells used for analyzing stable isotopes and essential fatty acids, along with regurgitated samples, will help further our understanding of diet composition in order to better answer these crucial questions.



## **Method validation and toxicological data for DSL-listed microbial substances for aquatic organisms (PO)**

TROTTIER, S.<sup>1</sup>, HARWOOD, M.<sup>1</sup>, FARLEY, G.<sup>1</sup>, FAILLE, C.<sup>1</sup>, BOUCHARD, P.<sup>1</sup>, LAUZÉ-MALOUIN, A.<sup>1</sup>, WALKER, B.<sup>1</sup>. <sup>1</sup>Quebec Laboratory for Environmental Testing, Environment Canada

Through CEPA (1999), there is a requirement for the risk assessment of all microbial substances that are new to Canada. However, this assessment framework does not include those microbial substances listed on the Canadian Domestic Substance List (DSL). The New Substances Program has committed to screen the Canadian Domestic Substance List (DSL) according to their ability to persist within aquatic and terrestrial environments as well as to assess pathogenicity and/or toxicity to aquatic and terrestrial organisms. As a result, the Quebec Laboratory for Environmental Testing (QLET) of the Science and Technology Branch of EC was asked to validate methodology and generate laboratory data on the toxicity of these microbial substances in aquatic environments.

## ***Sediment and Soil Toxicity Methods***

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### **Development of molecular tools for toxicant identification in sediment toxicity tests**

BAY, S.<sup>1</sup> and VULPE, C.<sup>2</sup>. <sup>1</sup>Southern California Coastal Water Research Project, <sup>2</sup>University of California

A potential new method for stressor identification in sediment toxicity tests is under development that is based on measuring changes in gene expression. Aquatic organisms produce distinctive patterns of gene expression in response to contaminant stress, and these patterns can be used in a diagnostic manner to investigate the cause of toxicity. A custom microarray containing approximately 8,000 probes for different gene transcripts was developed from the expressed genome of the estuarine amphipod *Eohaustorius estuarius*. Analysis of changes in amphipod gene expression using this microarray provides a large number of potential markers of stressor-specific responses that can be used to identify the presence and significance of contaminant and non-contaminant stressors. The results of initial studies to evaluate the ability of the *E. estuarius* microarray to distinguish different types of stressors will be presented. Changes in *E. estuarius* mRNA produced following exposure to model toxicants that include ammonia, copper, DDT, chlordane, and cyfluthrin were measured and used to identify a suite of diagnostic gene expression markers. The ability of these markers to identify unknown toxicants in blind samples will be tested. The genomic responses of *E. estuarius* to toxicants will also be compared to those of another standard test organism, *Daphnia magna*, exposed to a wide variety of chemical toxicants.

## **Improvements to Environment Canada's standard test method for *Hyalella azteca* (EPS 1/RM/33)**

MILLER, J. <sup>1</sup>, TAYLOR, L. <sup>2</sup> and SCROGGINS, R. <sup>2</sup>. <sup>1</sup>Miller Environmental Sciences Inc.,  
<sup>2</sup>Environment Canada

Since its publication in 1997, Environment Canada's (EC's) *Hyalella* test method, EPS 1/RM/33, has been used extensively for sediment toxicity testing in both federal and provincial programs. After 13 years of application, however, several technical advancements have been made which warrant review of the test method, updating of methodologies, and the publication of a 2nd edition. EC initiated a study in 2006 to improve culturing and testing methodologies and to come up with recommendations to be included in the test method revision. Research has focused on feeding rates (type of food and ration), age of test organisms and growth variability, light intensity for culturing, sediment:water ratios, a 14-day water-only exposure, and the evaluation of a reproductive endpoint. The results of this study and the scope of changes to EPS 1/RM/33 will be discussed.

## **More options and better choices: A new path for statistical analysis of sediment toxicity data**

VAN DER VLIET, L. <sup>1</sup>, ZAJDLIK, B. <sup>2</sup>, TAYLOR, L. <sup>1</sup> and SCROGGINS, R. <sup>1</sup>. <sup>1</sup>Environment Canada,  
<sup>2</sup>Zajdlik & Associates

Lab technicians know "the routine" for hypothesis-testing with continuous data such as growth: test for assumptions, click your way through an ANOVA, then proceed through a cornucopia of tests which are tailored for your experiment objectives. The decision trees are very similar between jurisdictions (EPA, Environment Canada, OECD), so we can be fairly confident that the scientific community is approaching consensus, at least on the most common testing scenarios. But what about comparing mortality (quantal) data between several test sites, as you would for a *Hyalella* test? The roads diverge: EPA favours the biomass approach or arcsin transformation; Environment Canada only provides options to test one sample with a control; OECD provides a longer list of possibilities. Environment Canada has recently revisited their guidance on analysis of quantal data from multiple (field) sites, with the goal of providing better statistical direction for standardized tests. We have developed two "streams" for quantal data analysis: (i) we advocate the use of logistic regression, followed by contrast testing, for the best analysis of quantal data; (ii) we allow the use of less-preferred (but readily available) tests, as a back-up option. Finally, we expect to make some changes to experimental design; these changes will ensure resources used in field collection and lab testing yield the best information, given practical constraints.

## **The cone test: Sediment toxicity testing, methods, and applications**

NORWOOD, W. <sup>1</sup>. <sup>1</sup>Environment Canada

The "cone test" was originally developed in 1997 in order to overcome the deterioration of overlying water quality that occurred during toxicity tests with benthic invertebrates in the standard "beaker test." At that time the recommended solution for this problem was the daily-renewal option in which overlay water is replaced at a rate of two volume additions/day either by the labour-intense manual method or with the expensive and possibly problematic

automated intermittent-renewal systems. Since its development, the cone method has been used extensively as a chronic toxicity test for site assessments, bioaccumulation analyses, and spiked sediment testing, and it has been successfully used with four different benthic invertebrates. The standard "cone test" method with *Hyalella azteca* will be described as well as modifications for specific types of tests, including 1-week bioaccumulation studies within a 4-week chronic test, organic contaminant testing, environmental metal mixture testing as well as tests with *Chironomus*, *Hexagenia*, and *Tubifex*.

#### **Influence of culture diet on toxicity of three reference toxicants to *Lumbriculus variegatus* and implications for bioaccumulation testing**

WATSON-LEUNG, T.<sup>1</sup>, KENNEDY, L.<sup>1</sup>, NAIDOO, J.<sup>1</sup>, MAHON, K.<sup>1</sup> and VAN GEEST, J.<sup>2</sup>

<sup>1</sup>Ontario Ministry of the Environment, <sup>2</sup>University of Guelph

At the Ontario Ministry of the Environment (MOE), in conjunction with the University of Guelph, a standardized test method for the assessment of bioaccumulation of contaminants from sediments into three aquatic organisms has been developed. The guidance in previously published bioaccumulation test methods with *Lumbriculus variegatus* offer suggestions in regard to culture diet—e.g., rabbit pellets (RP) or starter trout chow (TC)—but allow for flexibility in the method. The impact of three diets on reference toxicant tests with *L. variegatus* was assessed. At the MOE, *L. variegatus* cultures were maintained with the same feed rate of three different food types: TC, RP and a 50:50 mixture of TC and RP. Using 96-hour LC<sub>50</sub> tests (n = 15) it was determined that *L. variegatus* reared on TC were significantly more sensitive to potassium chloride (KCl) (0.80 g L<sup>-1</sup> (0.72 – 0.86 g L<sup>-1</sup>)) than were those reared on rabbit pellets (1.36 g L<sup>-1</sup> (0.88 – 1.72 g L<sup>-1</sup>)). The *L. variegatus* reared on the 50:50 diet were less sensitive to KCl than the TC-fed worms, and more sensitive than the RP-fed worms. Results from additional work on water-only reference toxicant testing with parachlorophenol and zinc sulphate, as well as bioaccumulation tests with field-collected contaminated sediments will be presented.

#### **An integrated testing program to assess nickel toxicity and bioavailability in freshwater sediments**

ROGEVICH, E.<sup>1</sup> and SCHLEKAT, C.<sup>1</sup> <sup>1</sup>Nickel Producers Environmental Research Association (NiPERA)

To assess nickel toxicity and behaviour in freshwater sediments, a large-scale laboratory and field sediment testing program was conducted. The program used an integrative testing strategy to generate scientifically-based threshold values for nickel in sediments and to develop an integrated equilibrium-partitioning-based bioavailability model for assessing risks of nickel to benthic ecosystems. The sediment testing program was a multi-institutional collaboration that involved extensive laboratory testing, field validation of laboratory findings, characterization of nickel behaviour in natural and laboratory conditions, and examination of solid phase nickel speciation in sediments. The laboratory testing initiative was conducted in three phases to satisfy the following objectives: 1) to evaluate various methods for spiking sediments with nickel to optimize the relevance of sediment nickel exposures; 2) to generate reliable ecotoxicity data by conducting standardized chronic ecotoxicity tests using nine benthic species in two sediment types with low and high nickel-binding capacity; and 3) to examine sediment bioavailability relationships by conducting chronic ecotoxicity-testing using

four benthic species in eight different sediment types. A subset of six nickel-spiked sediments was deployed in the field to examine benthic colonization and community effects. The sediment testing program yielded a broad, high-quality dataset which was used to develop a Species Sensitivity Distribution for benthic organisms in various sediment types, a reasonable worst-case Predicted No-Effect Concentration for nickel in sediment (PNEC<sub>sediment</sub>), and predictive model for bioavailability and toxicity of nickel in freshwater sediments.

### **Resolving false petroleum hydrocarbon detection issues in uncontaminated organic soils**

KELLY-HOOPER, F.<sup>1</sup> and DIXON, D.G.<sup>1</sup>. <sup>1</sup>University of Waterloo

Petroleum hydrocarbon (PHC) soil contamination is a globally recognized environmental issue. Contaminated site evaluations typically begin by analyzing soil samples in accordance with the Canada-Wide Standard (CWS) for PHC soil toxicity criteria. However, current PHC soil extraction and analysis techniques can misidentify natural biogenic organic compounds as originating from petroleum sources. "BOC" is a general term used to describe mixtures of biological organic compounds such as alkanes, sterols, fatty acids and wax esters. BOCs are biosynthesized by plants and animals during their life cycles and are integral components of naturally organic soils. Standard PHC analysis of highly organic soils such as peat can therefore trigger false PHC soil criteria exceedences. False exceedences can lead to unnecessary site remediation requirements, which can be financially costly as well as highly disruptive to functioning ecosystems. The University of Waterloo has developed a new approach to resolving false PHC detections in clean organic soils. This new approach was developed through a 300-day crude oil contamination experiment, a 300-day diesel fuel contamination experiment as well as field studies of 45 contaminated and uncontaminated sites located throughout Canada. The results of these studies demonstrate that carbon range distributions and Unresolved Complex Mixture (UCM) patterns can be used to quantify biogenic vs petrogenic compound concentrations in clean and contaminated soils. These evaluation techniques may be used to evaluate a wide range of PHC contamination scenarios in urban and natural environments.

### **Refined protocol development using *Lumbricus terrestris* to assess the potential impact of land-applied biosolids**

PUDDEPHATT, K.<sup>1</sup>, MCCARTHY, L.<sup>1</sup>, BOSTAN, V.<sup>1</sup> and LAURSEN, A.<sup>1</sup>. <sup>1</sup>Ryerson University

Incorporating a holistic approach is extremely important to assessing the potential impact of land-applied biosolids, and thus many environmentally-relevant bioassays have been implemented in our laboratory. *Lumbricus terrestris* was used because of its importance as an indigenous member of terrestrial biomes. Ecologically, it can be found in a variety of soil types, tolerates a broad range of pHs, and its natural habitat includes meadows, pastures, and open fields. These are the biomes of biosolids applications. Conversely, *Eisenia fetida* and *andrei* (Environment Canada RM 43) are found in organic-rich soil such as gardens, composts and manure piles. An important sub-acute bioassay examined the avoidance behaviours of *Lumbricus*. Environment Canada protocol RM 43 included the Kaushik chamber. Extensive testing in our laboratory indicated limitations to this design and we thus developed a chamber that was environmentally relevant, allowing the earthworm to completely elongate. For assessing impact on chronic and reproductive behaviours, an altered Evan's Box (Evans,



1947) was utilized. These acrylic, transparent chambers, with a vertical depth of 100 cm, allowed a natural and less stressful environment for the earthworms to perform their borrowing behaviours. Secondly, a modification was attached to the top of these Evan's Boxes to allow space for the *Lumbricus terrestris* to copulate above ground (their natural mating behaviour). These protocol refinements will be discussed in detail, including some preliminary results on potential biosolids impact.

#### **Development of a soil toxicity test using boreal wetland plants**

MOODY, M. <sup>1</sup>, GODWIN, B. <sup>1</sup> and SCROGGINS, R. <sup>2</sup>. <sup>1</sup>Saskatchewan Research Council, <sup>2</sup>Environment Canada

The development of methodology for testing contaminated soils using boreal forest plants as test species is nearing completion. The majority of the method development work has focused on demonstrating inhibition of growth of upland plants, though preliminary testing indicated that wetland plant species could be employed with minor modifications to the method. Since wetlands comprise approximately 16% of the land area of Canada, risk assessment tools for assessing contaminant-impacted wetland soils using ecologically relevant species is a priority. Three boreal forest wetland types were addressed: bogs, fens and shallow water wetlands/marshes. Candidate species were chosen for each habitat on the basis of criteria including frequency and abundance of species from a wide geographic area. Species representative of different growth forms (monocot herbs, dicot herbs, trees and shrubs) were included. Initial lists were refined on the basis of seed availability and germination following scarification and stratification. Species for each habitat will be tested to ensure sufficient growth in artificial soil at various test durations and in clean reference soils. Sensitivity to a range of pH levels, boric acid, and hydrocarbons or salts from spills of produced waters will further aid in selection of sensitive and ecologically relevant test species.

#### **New territory: Toxicity test methods to assess contaminated boreal forest soil**

PRINCZ, J. <sup>1</sup>, FRASER, C. <sup>1</sup>, MOODY, M. <sup>2</sup>, MALETTE, M. <sup>1</sup> and SCROGGINS, R. <sup>1</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>Saskatchewan Research Council

Due to significant industrial and commercial development in the boreal forest region, there is a recognised need for new standardized toxicity methods for the assessment of soil contamination from these industrial activities. Industry and regulatory authorities have recognized that there is a critical need for new test methods that use plant and invertebrate species representative of the boreal and taiga eco-zones of Canada. Existing soil toxicology methods published by Environment Canada use test species validated for soils of agronomic regions of Canada. New test methods are under development which better reflect the unique characteristics of the boreal forest ecosystem (i.e., stratified nature of boreal forest soils, difference in chemical characteristics such as depressed pH, species of plants and soil invertebrates native to forest soils). The current research is focusing on upland and wetland boreal plant species, and invertebrates (collembola and earthworms). This presentation will focus on the research conducted to develop test methods for the assessment of contaminated soils using 7 species of upland boreal plants, 2 boreal earthworm species, *Dendrodrillus rubidus* and *Dendrobaena octaedra*, the collembolan species, *Proisotoma minuta* and the oribatid mite species, *Oppia nitens*.

## **Protocol development of *Zea mays* and *Glycine max* to assess the potential impact of land-applied biosolids on terrestrial biota**

*PUDDEPHATT, K. <sup>1</sup>, MCCARTHY, L. <sup>1</sup>, BOSTAN, V. <sup>1</sup> and LAURSEN, A. <sup>1</sup>. <sup>1</sup>Ryerson University*

This study determined the impact, if any, of biosolids on the development and reproduction of *Zea mays* (corn) and *Glycine max* (soya bean), two crops of tremendous global importance. Unlike plants used in conventional protocols (Environment Canada RM 45) that are grown in 1L containers with 500 mL soil, our developed "crop troughs" measured 122x30x30 cm or 91x30x30 cm and contained 110 or 85 L of soil for corn and soya bean bioassays respectively. Additionally, while traditional plant protocols primarily measure germination rates, root and shoot lengths, and biomass at the completion of a 14- or 21-day partial lifecycle bioassay, we examined the entire life of the plant. Along with traditional observations, the shoot height, number of leaves, and area were also constantly measured throughout our experiment. Concurrently, we examined tassels and pods developed, their numbers and size, the number of seeds produced, as well as the biomass of the pods and seeds. Lastly, the germination rates of the seeds produced by the F<sub>0</sub> plants were examined. With these extra measurements taken throughout the 120-day bioassay, a more comprehensive assessment was made between the reference plants and the treatment plants throughout their entire lifecycle. As the need for a more holistic approach becomes necessary to accurately assess potential contaminant impact in all terrestrial ecotoxicity bioassays, we recommend that these methods be incorporated into the existing protocols of Environment Canada.

## **Environment Canada guidance on statistical sampling designs for the biological assessment of contaminated soils**

*ZAJDLIK, B. <sup>1</sup>, FEISTHAUER, N. <sup>2</sup>, VELICOGNA, J. <sup>3</sup> and SCROGGINS, R. <sup>3</sup>. <sup>1</sup>Zajdlik & Associates Inc, <sup>2</sup>Feisthauer Environmental, <sup>3</sup>Environment Canada*

The use of terrestrial toxicity tests with species intimately associated with soil has significant potential to augment the management of contaminated sites in Canada. Site-specific terrestrial toxicity testing provides data that integrate the toxicity of soil contaminant mixtures and the influence of soil characteristics on the bioavailability of contaminants and their effects on the survival, growth and reproduction of soil-dwelling organisms. Although Environment Canada has published terrestrial toxicity methods for earthworms, plants and collembola testing, what is lacking is comprehensive guidance on the sampling, collection, handling and preparation of contaminated and reference soil for biological testing. In 2007, Environment Canada initiated a project to develop a new guidance document for the sampling, collection, handling and preparation of soil for biological testing. A component of this new EC document is guidance on choosing appropriate statistical designs for sampling soil at contaminated sites for the purposes of biological testing. An outline of the statistical methods will be presented. Implications for contaminated site managers, consultants, laboratories and site risk managers involved with assessing and remediating the effects of contaminants in soils will be discussed.



## **Bioavailability and bioaccessibility of petroleum hydrocarbons in contaminated site soils**

STEPHENSON, G. <sup>1</sup>, ANGELL, R. <sup>1</sup>, SHRIVE, E. <sup>1</sup> and MA, W. <sup>1</sup>. <sup>1</sup>*Stantec Consulting Ltd.*

There are a number of methods with which to measure the bioavailability and/or bioaccessibility of contaminants in soil by ecological receptors. However, the methods that work well for metals do not necessarily work well for petroleum hydrocarbons. We applied a number of methods (biological and chemical) to different types of site soils contaminated with petroleum hydrocarbons (PHC) to determine which method might be most suitable for which soil type in terms of predicting the biological responses of organisms as measured by standard single species toxicity tests. Organisms (plants, earthworms, and collembolan) were exposed to soils with different PHC concentrations, and multiple endpoints were measured to assess the biological responses (e.g., effects). The exposure concentrations for the four CCME hydrocarbon fractions were measured using the standard CCME analytical method (hexane:acetone extraction) as well as extractions with cyclodextrin, and a mixture of enzymes to simulate the gastro-intestinal fluid of an earthworm. The different extraction methods affected the estimated exposure concentrations and differed in the degree to which they correlated with the biological responses. Modifications of the existing methodologies were required in order to improve the estimates of the effect concentrations for petroleum hydrocarbons in soil. Comparative data will be presented and discussed along with the proposed methodological modifications required for PHCs.

## **Assessing fresh and weathered petroleum hydrocarbon (PHC) fraction 2 direct soil contact values for ecological receptors**

ANGELL, R. <sup>1</sup>, KULLMAN, S. <sup>2</sup>, SHRIVE, E. <sup>1</sup>, TINDAL, M. <sup>3</sup> and STEPHENSON, G. <sup>1</sup>. <sup>1</sup>*Stantec Consulting Ltd.*, <sup>2</sup>*Husky Oil Operations Ltd.*, <sup>3</sup>*Axiom Environmental Inc.*

Ecological Tier 1 Canada-wide standards (CWS) for petroleum hydrocarbon (PHC) Fraction 2 (>nC10 to C16) in soil were derived using ecotoxicological assessment endpoints (EC/LC/IC25s) with freshly spiked ("fresh"), fine-textured soils. These soil standards might be needlessly conservative when applied to field samples with weathered hydrocarbons. The purpose of the present study was to assess the toxicity of weathered PHC Fraction 2 (F2) in soil and derive direct soil contact values for ecological receptors. Fine-textured soils were spiked with distilled F2 and weathered for six months. Toxicity tests using plants and invertebrates were conducted with the weathered F2-spiked soils. Endpoint EC/IC25s were calculated and used to derive soil standards for weathered F2 in fine-grained soil protective of ecological receptors exposed via direct soil contact. Current ecological Tier 1 CWS for fresh F2 in soil were derived using EC/LC/IC25s predicted from the relationship between nominal and measured F2-spiked low, medium, and high concentrations in soil, which was assumed to be linear. The values derived for weathered F2 are less restrictive than current ecological Tier 1 CWS for F2 in soil, but values for fresh and weathered F2-spiked soils are similar when current CWS are adjusted using a non-linear, more accurately predictive regression ( $y = ax^b$ ). The soil contact values for fresh F2 in fine-textured soil would be ~2× higher if current ecological Tier 1 CWS were adjusted using a non-linear relationship to increase predictive accuracy.

## **Application of boreal forest toxicity data in the decision-making process for contaminated soil clean-up remediation at oil and gas fields in Western Canada**

SCROGGINS, R. <sup>1</sup>, PRINCZ, J. <sup>1</sup>, MOODY, M. <sup>2</sup>, OLSGARD-DUMANSKI, M. <sup>3</sup>, HADERLEIN, L. <sup>3</sup> and MOORE, B. <sup>4</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>Saskatchewan Research Council, <sup>3</sup>WorleyParsons Canada, <sup>4</sup>Devon Corporation Canada

Using a battery of boreal forest test methods for assessing petroleum hydrocarbon (PHC) toxicity in contaminated soil, our multi-year research project hopes to demonstrate that clean-up decisions can be made on a field-wide basis through focused biological testing of typical drill sump and flare pit locations within an oil and gas field. It is anticipated that remediation at the majority of the sites will be limited exclusively to the Alberta soil eco-contact guidelines for PHC F2 and F3 fractions. As the Tier 1 eco-contact guidelines were derived using toxicity data from fresh crude and using agricultural plant species, it is more appropriate to follow a Tier 2 eco-contact pathway approach, as the majority of contamination is related to drilling sumps and flare pits containing highly weathered and/or aged PHCs and species native to the boreal eco-zone of Canada. Given the large number of sites requiring remediation and the similarity of sites within pre-determined Risk Assessment Zones, the site-specific remedial objective (SSRO) option within the Tier 2 guideline is being used. For representative contaminated soils, a SSRO is derived from the 25<sup>th</sup> percentile of the estimated species sensitivity distribution of all acceptable boreal plant, earthworm, springtail and mite test endpoints. The project is attempting to reduce soil volumes sent to landfill during site remediation by demonstrating that residual impacts from weathered PHC in soil (which exceed Tier 1 soil eco-contact guidelines) do not have deleterious effects on boreal forest receptors following remediation (in land treatment cells or remedial excavations). Data will be presented to demonstrate the utility of this approach, with the goal of understanding the sources of variability between sites and their effect on regionalizing a Tier 2 eco-contact guideline.

## **Methodological framework for establishing toxicity-based site-specific remedial objectives for contaminated soils**

ZAJDLIK, B. <sup>1</sup>, STEPHENSON, G. <sup>2</sup>, RENOUX, A. <sup>3</sup> and MOULINS, L. <sup>4</sup>. <sup>1</sup>Zajdlik & Associates, <sup>2</sup>Stantec Consulting Ltd, <sup>3</sup>Sanexen Services Environnementaux Inc., <sup>4</sup>Xstrata Copper Canada

Site-specific toxicity tests with soils containing contaminant mixtures can be applied strategically to a contaminated site such that site-specific remedial objectives can be derived and risk estimated. Inherent in this approach is less uncertainty relative to a generic approach which relies on toxicity data available from the literature. Surface soils from 43 locations on a site contaminated with metal mixtures were collected. Standard methods were used to assess soil toxicity to plant and invertebrate species. The sampling strategy was to obtain soils with a range of metal concentrations in order to establish relationships between the presence of metals in soils and the biological responses as reflected by multiple measurement endpoints. The toxicity test results indicated that both growth of plants and growth and reproduction of the invertebrates were affected by the quality of the soils. Relationships between the biological responses and soil physical-chemical analytical suite were modelled using generalized linear models. Because the distributions of the metals were inter-correlated on the site, 1) synthetic variables describing the correlates were used as

surrogate independent variables; and, 2) this observational study could not establish metal-specific causality. However, it was possible to develop predictive tools for the metals mixture comprising the contamination at the site. These regression equations could then be adjusted for pedological parameters and when accompanied by acceptable effect thresholds, used to determine site-specific remedial objectives for the contaminants of concern.

#### **Use of an oiled gravel column dosing system to characterize exposure and toxicity of fish to sunken heavy oil on spawning substrates (PO)**

MARTIN, J. <sup>1</sup> and HODSON, P. <sup>1</sup>. <sup>1</sup>Queen's University

Nearly 150 000 L of No. 6 heavy fuel oil (Bunker C) was spilled into Wabamun Lake (Alberta, CA) when a train derailed in August 2005. Bunker C is a residue of refining crude oil and a very complex mixture containing polycyclic aromatic hydrocarbons (PAH), particularly the 3-4 ringed alkylated forms that cause sub-lethal toxic responses in early life stages of rainbow trout (*Oncorhynchus mykiss*). Static daily renewal tests with heavy fuel oil coated on glass plates (artificial substrates) demonstrated greater oil toxicity (as mg added oil L<sup>-1</sup>) to trout embryos than oil that was mechanically or chemically dispersed. Nearly four years after the spill at Wabamun Lake, oil patches still persist in near-shore sediments where fish spawn. The toxicity of oil that sinks and persists in sediments is not well understood because the mechanisms of exposure are not characterized. The objective of this research is to assess how the behaviour of heavy oil in water interacts with exposure and toxicity to early life stages of fish. Specifically, flow-through oiled gravel columns were used to assess whether the toxic constituents of heavy oil are transferred to water quickly enough to cause toxicity. Dose-dependent mortality and signs of sub-lethal toxicity were observed in embryonic trout exposed to the outflow of these columns. As well, column output of hydrocarbons and CYP1A induction in fish were found to be flow-dependent. To assess the toxicity of oil on these substrates and relate it back to toxicity of oil in sediments, there was a need to characterize the desorption kinetics of the gravel column dosing. This involved determining the time to steady-state desorption of oil constituents in water and identifying the rate at which different classes of oil constituents partition into water. Overall, this research will help to define the toxic load of oil in sediments to better describe the exposure and toxicity of fish to sunken heavy oil on spawning substrates.

#### **Ah-receptor-agonist activity of sediment extracts and native sediments from Hamburg Harbor and the Rhine River in *Danio rerio* (PO)**

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The European Water Framework Directive (EWFD) aims to achieve a good ecological and chemical status in the surface water of European rivers until 2015. However, there is still need for basic research in order to fulfill this legal obligation. Sediment toxicology plays a major role in this intention as sediments can act as a secondary source of pollution. The present study is part of the joint research project DanTox, which – among other specific endpoints – investigates dioxin-like effects of acetonic sediment extracts from the Rhine River and Hamburg Harbour in zebrafish (*Danio rerio*) embryos. In the present study, the fish embryo toxicity test (FET) and the 7-ethoxyresorufin-O-deethylase (EROD) assay were combined:

Embryos were exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or acetonic sediment extracts for 48 h according to the FET (DIN 38415-6). Subsequently, embryos were homogenized and used in the EROD assay. The induction of EROD indicates dioxin-like activity of environmental samples on the test species. Exposures to TCDD lead to a decrease in EROD activity below basal level while exposures to acetonic sediment extracts lead to inductions above basal level and tendencies towards dose-response relationships could be seen. The fish egg EROD assay has shown to be an applicable test to investigate dioxin-like activity in *Danio rerio* embryos; however, TCDD does not seem to be suitable as a positive control since the results indicate that the natural barrier function of the chorion prevents TCDD from entering and harming the embryo. The RWTH Aachen University Undergraduate Funds, as part of the German Excellence Initiative provided funding for participating in the ATW meeting by a personal travel grant to the first author.

### ***Pesticides in Urban Environments***

#### **Effects of atrazine on phytoplankton and periphyton in freshwater mesocosms and interactions with phosphorus**

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Recent studies have investigated the potential role of the herbicide atrazine in the rise of limb-deforming parasite infections in amphibians. The proposed hypothesis suggests that atrazine indirectly enhances growth of periphyton, a preferred food source for the aquatic snails that act as the parasite's first larval host. Concurrently, evidence has found that high nutrient inputs may trigger the same chain of effects. Since both atrazine and nutrient loadings commonly enter surface waters from agricultural run-off, their spatial and temporal co-occurrence are highly probable in affected rural regions. Therefore it is important to characterize this relationship under environmentally relevant conditions, and to determine which stressor is the key driver. In the summer of 2009 an outdoor mesocosm study was conducted to examine the relationship between atrazine, algal biomass and snail populations over a range of atrazine concentrations (0, 1, 10, 30, and 100  $\mu\text{g L}^{-1}$ ;  $n=3$ ). Over 70 days, measures were taken of phytoplankton, periphyton and snail population parameters. No significant effect of atrazine on algal biomass or snail populations was found. A further 70-day mesocosm study in 2010 examined responses of phytoplankton, periphyton and snail growth in simplified systems (absence of macrophytes and sediment) to manipulations of atrazine and total phosphorus concentrations using a central composite rotatable design (0-200  $\mu\text{g L}^{-1}$  atrazine; phosphorus additions of 0-200  $\mu\text{g L}^{-1}$ ). Preliminary analysis of these two studies supports an interaction between phosphorus and atrazine for algal growth but no effect at the next trophic level studied (snails). Study design, experimental methods and results will be discussed.



## **New developments in passive air sampling for current-use pesticides**

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Passive air samplers are cost effective and simple tools that are invaluable for assessing risks associated with pesticide use. They can be used to monitor and evaluate regional and long range transport of pesticides and provide spatially resolved data that is required for developing transport and fate models. Under the Global Atmospheric Passive Sampling (GAPS) network, passive air samplers comprising polyurethane foam (PUF) disks are being used to deliver air concentrations of several organochlorine pesticides at more than 50 global sites. The value of these samplers has been recognized and there is a need to extend their application to more volatile and polar pesticides that are typically used these days. This study investigates a modified PUF disk sampler, the SIP (or sorbent-impregnated PUF) disk which used XAD powder impregnated into the PUF to enhance sorptive capacity. A calibration study of the PUF disk and SIP disk samplers has been undertaken (March-October 2010 in Downsview) to establish sampling rates and sorptive capacities for a range of current-use pesticides that are relevant to Canada. Preliminary results are also available from the first deployment of the SIP disk sampler at a subset of 20 GAPS sites alongside the conventional PUF disk sampler. This study provides the first global-scale survey of several classes of priority chemicals, including CUPs. GAPS network covers also several urban sites (e.g. Toronto, Canada; Paris, France). These locations can be compared to background and agricultural areas and this may provide useful information about transport of CUPs from application areas.

## **The acute and chronic effects of pulse-dosed endosulfan on *Jordanella floridae* (Florida Flagfish) over one complete life-cycle**

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Endosulfan is a commonly used organochlorine in Durham Region, Ontario Canada which has known toxic effects on non-target organisms including fish. This research investigated the acute and chronic effects of endosulfan on Florida flagfish (*Jordanella floridae*), using pulse-exposure. A 4-h pulse-exposure of endosulfan to larval flagfish caused a significant increase in mortality after 96-h at nominal concentrations equal to or greater than 100 µg L<sup>-1</sup>. Some of the acute sub-lethal observations included hyperactivity, convulsions, and axis malformation. A 4-h pulse-exposure of endosulfan to 7-8 days old post-hatch flagfish was monitored for chronic effects in growth, reproduction, and survivability over one full life-cycle. There were no growth or reproductive effects of endosulfan pulse-exposure up to the highest exposure concentration of 10 µg L<sup>-1</sup>. Thus, the life-cycle 4-h pulse-exposure no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were 3.2 and 10 µg L<sup>-1</sup> endosulfan, respectively, due to significantly higher mortality.

## **Monitoring changes in urban stream water pesticide concentrations following a cosmetic pesticides ban**

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On April 22, 2009, an Ontario ban on the sale and use of pesticides for cosmetic purposes took effect. Stream water pesticide concentrations in ten urban Ontario watersheds were monitored before (summer 2008) and after (summer 2009) the ban to assess changes. Samples were collected approximately biweekly between June and October and analyzed for up to 105 pesticides and degradates. Herbicides 2,4-D, dicamba, glyphosate and MCPP and the insecticide carbaryl were the dominant pesticides in 2008 samples. Cosmetic uses of these pesticides were banned and, with the exception of glyphosate, sales of domestic products containing these pesticides were prohibited when the ban took effect. Continued purchase and use of glyphosate is allowed under the ban to control plants poisonous to the touch. Concentrations of 2,4-D, dicamba and MCPP were significantly lower in 2009 and a decrease in carbaryl concentrations approached statistical significance; however, concentrations of glyphosate and its degradate AMPA were unchanged. Rainfall was similar in the summers of 2008 and 2009 suggesting that changes in pesticide use, and not changes in runoff, were responsible for the observed changes in stream water pesticide concentrations. The study is ongoing and preliminary results from 2010 samples may be available at the time of this presentation.

## **Evaluating the cytotoxic effects of organophosphate pesticides using fish cell lines derived from neural and non-neural tissues**

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Organophosphate (OP) pesticides have been shown to inhibit the proper functioning of nerves, specifically targeting the enzyme acetylcholinesterase (AChE). OP pesticides are thus neurotoxic and are highly toxic to insects as well as to humans and other non-target organisms like fish. Although not as persistent as the organochlorine pesticides, in most cases OP pesticides have been shown to have greater acute toxicity. With the liberal use of these compounds in agricultural practices many organisms are at risk of coming into contact with OP pesticides for example, through agricultural runoff into aquatic environments. Toxicity evaluation in whole organisms, especially in fish that need large wet laboratory facilities can be costly, thus alternative in vitro models are being sought. In the present study, we report on the effects of OP pesticides both to cell viability and AChE activity using cell lines derived from neural and non-neural tissues of trout, goldfish and eel. We have optimized Ellman's AChE assay for rapid high throughput analysis using a multiwell spectrophotometric plate reader and compared responses to four OP pesticides: guthion (azinphos-methyl), malathion, parathion and disulfoton. Highest AChE activity was recorded with EelB cells (derived from Eel brain) followed by RTB (Rainbow trout brain) and GF3BC (derived from goldfish cerebellum). Non neural tissue derived cells like RTgill-W1 (trout gill) and GFSk-S1 (goldfish skin) had low AChE activity. In terms of inhibition of AChE activity, parathion was more active than disulfoton and RTB was more sensitive than GFB3C or EelB. This was also the trend seen in terms of cell viability as measured using Alamar Blue. Parathion exhibited significantly higher toxicity to RTB with respect to GFB3C or EelB. The sensitivity of the cell lines reflect the



sensitivities of whole fish, with rainbow trout being quite sensitive to environmental contaminants, while eel and goldfish were more tolerant.

### **Pesticides in surface waters of Lake of the Woods (PO)**

*MCDANIEL, T. <sup>1</sup>, PASCOE, T. <sup>1</sup> and STRUGER, J. <sup>1</sup>. <sup>1</sup>Environment Canada*

Lake of the Woods is a large, multi-basin water body, on the borders of Ontario, Manitoba and Minnesota, in the Lake Winnipeg basin. Concerns regarding excessive nutrient loading and algal blooms prompted the formation of Environment Canada's Lake of the Woods Science Initiative to explore nutrient loading and water quality issues in the lake. In June and September of 2009 surface water samples were collected from 8 stations on Lake of the Woods and analyzed for herbicides including glyphosate, sulfonyl urea herbicides, triazine herbicides and metolachlor. Atrazine, 2,4-D, dicamba, diuron and thifensulfuron were all present in surface waters of at least some of the stations on Lake of the Woods. In general, herbicide concentrations in Lake of the Woods were low in comparison to those measured in southern Ontario surface waters and were well below Canadian Water Quality Guidelines for all compounds measured. This is likely reflective of the relatively small proportion of land used for agriculture in the Lake of the Woods watershed.

## ***EEM for Mining in Canada***

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### **National patterns of effects in the metal mining Environmental Effects Monitoring Program: Relationships to potential influencing factors**

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<sup>1</sup>Environment Canada*

The National Environmental Effects Monitoring (EEM) Program assesses the effects of metal mining effluents on receiving waters across Canada. Over the first two phases of the program, a number of potential effects were measured in effluent exposed fish and benthic invertebrate communities. When effects occurred, they were typically inhibitory, although stimulatory effects were also observed. Examples of inhibitory effects included decreases in fish condition, relative liver size, and/or growth rate, and decreases in invertebrate taxon richness. The cause(s) of these effects is an area of active investigation, with detailed studies being done on a site-specific basis. On a more broad scale, national patterns of effects were analyzed to determine the relationship between effect magnitudes and potential influencing factors. For the most part, effects were fairly consistent from the first to second phase of the EEM program, although some shifts in response patterns between phases were also observed. These shifts were analyzed to determine where they occurred with respect to certain habitats or ore types. Changes in effluent flow rates were also compiled and investigated for possible correlations to the shifts in effect magnitudes.

## **Overview of EEM in mining sector, Quebec Region: What benthos tells us about water quality?**

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As part of Environment Canada's Environmental Effects Monitoring (EEM) program, metal mines discharging an effluent into the aquatic environment are required to conduct biological monitoring studies in order to evaluate their effects on fish, fish habitat and the use of fisheries resources.

A summary is presented of the first two phases of data from the benthic invertebrate community surveys submitted by mines located in Quebec. Effects on benthic communities are measured by comparing univariate descriptors such as density, taxon richness, evenness and Bray-Curtis index between exposure and reference areas. Data from 37 studies (21 in Phase 1 and 16 in Phase 2) show that the overall pattern after two phases in Quebec is an increase in benthic density, a decrease in richness and an increase in Bray-Curtis. These could be interpreted as preliminary signs of eutrophication, mixed with some toxic effects, as shown at some specific mines.

In order to verify if there were links between benthos results and water quality in receiving environments, a discriminant analysis was performed. The results show that Bray-Curtis dominates the canonical axis #1. Therefore, differences between exposure and reference areas are mainly explained by Bray-Curtis index. Another discriminant analysis was then run, without the Bray-Curtis index. In this analysis, hardness and nitrates were responsible for the discrimination between the two areas. Nitrogen compounds and hardness could explain the effects observed on benthic communities in Quebec as shown by the differences measured with the Bray-Curtis index.

Two case studies are presented: Laronde and Niobec mines. These showcase the impact of the effluent on the receiving environment.

## **EEM: An outsider's perspective**

*PAQUETTE, C. <sup>1</sup>, HUEBERT, D. <sup>2</sup> and MCKERNAN, M. <sup>2,1</sup>University of Manitoba, <sup>2</sup>Stantec Consulting*

The Environmental Effects Monitoring (EEM) program has been in place since 2002, when the Metal Mining Effluent Regulations were registered. The purpose of the EEM guidance document as written was, "to evaluate the effects of mine effluent on the aquatic environment", and was designed to achieve "national uniformity" in monitoring, and "to provide guidance on recommended standards of good scientific practice...". Within the EEM guidance document, however, it was recognized that updates would be required as new information became available. The purpose of this study was to evaluate the EEM guidance document against recognized standards of good scientific practice. Despite considerable effort, there are currently at least three serious methodological and/or statistical deficiencies within the EEM. First, the basic study design is pseudo-replicated. The result is that alpha is undefined and the study is confounded; differences between reference and impacted sites could be due to natural variability, or effluent impacts. Second, notwithstanding the first point, the probability of a Type 1 error is misrepresented, and is not 10% as stated, but actually over 60%. Third, calculation errors introduced into development of the Bray-Curtis

Index result in a bias towards finding an 'effect' under almost any ecological circumstance. Together, these three deficiencies result in an almost certain finding of 'effect', even in the absence of environmental 'impact'. The resultant requirements for further monitoring, and ultimately demands for corrective action to address the perceived threats to the environment, will potentially and unnecessarily cost mining and forestry companies millions of dollars and exposure to legal liability under the federal *Fisheries Act*. Proposals for creating remedy for these deficiencies will be presented.

#### **An assessment of environmental effects monitoring program endpoints in a baseline environment: Are effects found prior to mine effluent release?**

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<sup>1</sup>Canada North Environmental Services, <sup>2</sup>Cameco Corporation

Cameco Corporation, and its joint venture partners (AREVA and JCU), have recently completed a pre-feasibility study for the proposed development of an underground uranium mine at the Millennium deposit located in northern Saskatchewan. As part of the studies conducted to characterize the existing environment at the Millennium site, an Environmental Effects Monitoring (EEM) based program was performed to establish a baseline for the site's future EEM studies. Benthic invertebrate community and fish population surveys were conducted at the proposed future "exposure" site in Moon Lake and at a "reference" site in Slush Lake. Site characterization information demonstrated that Slush and Moon lakes have similar habitats, water and sediment chemistry, and fish communities. The results of the EEM based survey found effects, as defined by the EEM program, on fish and the benthic invertebrate community in Moon Lake. All EEM effect endpoints for the benthic invertebrate community survey were significantly different between the study areas. Results from the northern pike non-lethal fish population survey showed that young-of-the-year fish were significantly larger in Slush Lake than in Moon Lake. The lethal yellow perch study found that male fish from Moon Lake had significantly larger size-at-age, while female fish from Slush Lake had significantly larger relative gonad and liver sizes. These results show that metal mining EEM defined effects (i.e., statistically significant differences) can occur naturally in the environment between two similar un-impacted waterbodies. This stresses the importance of using multiple reference sites and using an appropriate effect size for determining mine-related impacts since statistical differences do not equate to ecological effects.

#### **Examining waterborne and dietborne routes of exposure and their contribution to biological response patterns in fathead minnow (*Pimephales promelas*) exposed to metal-mining effluents**

DUBÉ, M. <sup>1</sup>, NIYOGI, S. <sup>1</sup>, SQUIRES, A. <sup>1</sup> and RAMILO, L. <sup>1</sup>. <sup>1</sup>University of Saskatchewan

The objectives of the current study were: i) to gain a better understanding of the relative importance of water and diet as routes of exposure causing toxicity in fathead minnow (FHM) exposed to metal-mining effluents (MME) using a full factorial water/food experimental design (Exposure 1), and ii) to assess differences in the effects of food quality on toxicity by comparing FHM fed both a live and frozen diet of *Chironomus dilutus* (Exposure 2). The results showed significant increases in general water quality parameters (e.g., hardness, conductivity) and various metals in the effluent treatment waters compared to control waters, with maximum increase seen in the multi-trophic streams. Metals accumulation (Rb, Al, Se, Sr, Tl,

Ce, Co, Cu, Pb) effects of both waterborne and multi-trophic exposures were significant in one or more fathead minnow tissue type (muscle, gonads, liver, larvae) relative to those in the control systems. Condition factor and liver somatic index (LSI) of FHM were also significantly affected in both experiments by one or both routes of exposure (water and/or diet). In addition, cumulative total egg production and cumulative spawning events were significantly affected by both waterborne and dietborne exposures, with maximum effect found in the multi-trophic streams. These results suggest that under environmentally relevant exposure conditions, trophic transfer of metals may lead to greater reproductive effects and increased metal toxicity in fish. It also indicates that metals are assimilated in tissues differently depending on the quality of the food (live versus frozen).

#### **A multi-trophic approach to assess the effects of a complex uranium milling effluent on fathead minnow (*Pimephales promelas*) reproduction and survival**

DRIESSNACK, M. <sup>1</sup>, JONES, P. <sup>1</sup> and DUBÉ, M. <sup>1</sup> *University of Saskatchewan*

Industrial activities in Canada associated with metal mining operations such as uranium have contributed to increased levels of metals in aquatic environments. Here a fathead minnow (*Pimephales promelas*) partial lifecycle reproduction assay was used to evaluate the effects of exposure to a uranium milling effluent, elevated in selenium (Se), through a multi-trophic application. Treatments were reference, 5% and 25% diluted effluent, and 1% effluent with Se as selenate added to 120% whole effluent concentration and 10% effluent with 500% Se. Mesocosm tables were inoculated with algal/biofilm samples collected from site, as well as seeded with lab reared *Chironomus dilutus*, to provide dietary exposure. During the course of the study, fish survival (adult and 5 day larval), larval deformities, reproductive effects (egg production, spawning events) as well as metal tissue burdens and Se speciation (muscle and gonad) in female fathead minnows were evaluated. In addition, to the exposure phase there was a subsequent 21-day depuration-like phase with three of the initial eight fathead minnow breeding pairs per treatment. Egg production showed significant decreases within the exposure and depuration phases and between phases as well as changes in larval deformities, survival, larvae size and egg size. Spawning had ceased in the 500% treatment and was significantly decreased in 120% during the depuration phase. There were significant increases in some metals, (i.e. Cd, As, Mo, Rb, Se, and U) in the female fathead muscle, ovaries, eggs, larvae, biofilms and invertebrates during the exposure phase with evidence of significant reductions in values following depuration in the biofilms, with no change in the invertebrate or fish tissues. This study intends to provide a clearer understanding of the dynamic nature of a complex effluent through a system with a specific emphasis on Se speciation, transport and modification.

#### **Evaluating multiple-stressor interactions in cumulative effects assessment**

SOMERS, K. <sup>1</sup>, SARRAZIN-DELAY, C. <sup>2</sup> and KELLER, W. <sup>2</sup>. <sup>1</sup>Ontario Ministry of the Environment, <sup>2</sup>Laurentian University

Quantitatively evaluating the cumulative effects of multiple stressors involves estimating the individual and combined effects of two-or-more stressors. Individual effects are relatively easy to measure, whereas quantifying combined effects is more challenging. Previously we have used redundancy analysis to estimate the redundant or overlapping effects of two sets of stressors. However, that type of analysis does not distinguish the multiplicative interaction



between multiple stressors. We use a hypothetical scenario based on the Environment Canada Environmental Effects Monitoring Program to show how multiplicative interactions can be quantified using traditional redundancy analysis or variance partitioning analysis. Downstream variation in four benthic community metrics is partitioned between pulp-mill and metal-mining point source effluents and their interaction. This approach further quantifies the individual and combined effects of multiple stressors that potentially affect the benthic community, providing an improved quantitative tool for cumulative effects assessment, and permitting an objective evaluation of the separate and combined impacts of multiple stressors.

#### **Investigating causes of metal mine effluent reproductive effects on fathead minnows (*Pimephales promelas*) using a single metal vs whole-effluent approach**

OUELLET, J. <sup>1</sup>, DUBÉ, M. <sup>1</sup> and NIYOGI, S. <sup>1</sup>. <sup>1</sup>University of Saskatchewan

Treated metal mine effluents (MMEs) are discharged into waterways and may affect invertebrate and fish populations in their receiving environments. Metals, such as copper, nickel, and selenium are suspected to be involved in causing reproductive impairment in MME exposed fathead minnows (*Pimephales promelas*). Past research has identified impacts from mining discharges; investigating causes of these impacts is the next step towards improving effluent treatment. Twenty-one day multi-trophic exposures were performed using fathead minnows to compare reproductive effects of effluent-matched single metal exposures of copper, nickel, and selenium to reference water, as well as to environmentally relevant concentrations of MME from a northern Ontario metal mine. Cumulative egg production was found to decrease in the copper treatment relative to the reference (-34.2%) (two-sample Kolmogorov-Smirnov;  $p < 0.05$ ), while there was no statistical difference between the MME and reference groups. Copper was also found to bioaccumulate from biofilm to Chironomids which was associated with an increase in copper levels in the livers of fathead minnows in both MME and copper-only treatments. Cumulative egg production in the nickel exposure was also lower than the reference (-21.7%), while the MME was significantly lower than the reference, nickel, and selenium treatments (two-sample Kolmogorov-Smirnov;  $p < 0.05$ ). These results indicate that copper likely plays an important role in producing MME effects in fish, while nickel may also impact reproduction. Future work will examine whether water chemistry modifications can reduce the toxicity of these metals and the MME.

#### **Investigation of Cause for MMER using *Hyalella azteca*: Results from the Birchtree Mine**

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When consistent effects of mine effluent discharge are identified in successive EEM cycles and the geographic extent of the effects are known, the mine is required to undertake Investigation of Cause. At the Vale Inco Birchtree nickel mine near Thompson, MB, effects on benthic invertebrate community structure were identified in two successive cycles. As an investigation of cause study, Stantec proposed to measure the concentrations of trace metals in *Hyalella azteca* tissues. The lethal body concentrations at which 25% mortality of exposed *Hyalella* will occur (the LBC25) of a number of trace metals are published. Analysis of composite tissue samples of field-collected amphipods showed that concentrations of most trace metals were lower in animals collected from the exposure area than in animals collected



from the reference area, likely due to higher calcium concentrations in the exposure area, also originating from the mine effluent, which would tend to reduce uptake of those metals. Two trace metals for which critical body burden values are available (cobalt and nickel) showed higher concentrations in amphipods collected from the exposure area. The difference was small in the case of cobalt, and the measured concentration in *Hyaella* from the exposure area was less than 20% of the LBC25 value. For nickel, amphipods from the exposure area exhibited tissue concentrations ( $11 \text{ mg kg}^{-1}$ ) that were 96% of the LBC25 value ( $11.4 \text{ mg kg}^{-1}$ ), and higher than the concentration measured in the reference area ( $5 \text{ mg kg}^{-1}$ ). The mine complies with the effluent quality requirements of the MMER, however, nickel concentrations were higher in water samples collected from the exposure area than in samples from the reference area. Based on a weight of evidence it is considered likely that exposure to nickel is responsible for alteration of the benthic invertebrate community in the exposure area.

### **Determining the effect of a reduced YCT diet on the standard 7-day *Ceriodaphnia dubia* sub-lethal toxicity test (PO)**

KING, M. <sup>1</sup>, RICKWOOD, C. <sup>1</sup> and CAMERON, B. <sup>1</sup>. <sup>1</sup>Natural Resources Canada

Research to develop predictive models for metal toxicity have highlighted that the food required to conduct sub-lethal tests can greatly reduce metal and effluent toxicity. This is especially true for metals with a binding affinity for organic matter such as copper. Currently, the 7-day *Ceriodaphnia dubia* sub-lethal toxicity tests uses a diet composed of equal amounts of YCT (yeast, cerophyll and trout chow) and algae. Consequently, the YCT, a source of organic matter, may be interfering with the toxicity of specific elements during these 7-day tests. Therefore, our goal was to reduce the volume of YCT (if not eliminate) in their diet. Trial culture tests were conducted with different volumes of YCT-to-Algae ratios to determine a healthy culture suitable for toxicity testing. In addition, side-by-side 7-day toxicity tests with both copper and zinc were performed using the normal feeding regime and alternate diets with reduced YCT and algae-only. Preliminary results for zinc toxicity tests were found to be inconclusive for the algae only tests; however, copper toxicity results were significantly altered when YCT was removed from the *C. dubia*'s diet. More sensitive EC<sub>50</sub> and IC<sub>25</sub> were found when YCT was absent from the culture's diet, indicating that the organic matter may have had an effect on the copper's toxicity. Further testing is required to determine if this effect may have an impact on effluent monitoring.

## ***Integrating Ecosystem Health & Management***

### **Assessment of mercury near a chlor alkali facility**

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Total mercury levels (THg) in fish, sediment and aquatic plants taken from Sagua la Grande River (Cuba) in the vicinity of an active chlor-alkali plant (CAP) were assessed to evaluate the possible health risks from fish consumption. The study also aims at guiding

public health officials to establish regulations on fish consumption from the Sagua la Grande River. The average of THg in fish was  $0.33 \pm 0.05 \mu\text{g g}^{-1}$ , but never exceeding the Cuban guideline for fish consumption of  $0.50 \mu\text{g g}^{-1}$  ww. All sediments were highly contaminated with THg concentrations of 3 to 1400 times higher than the threshold effects levels (TEL) of  $0.174 \mu\text{g g}^{-1}$ . THg levels were significantly higher in *Eichhornia crassipes* (Water Hyacinth), particularly in the plants that were taken downstream of the CAP, suggesting a connection between mercury bioaccumulation and plant discharges. A Food Frequency Survey done in the population of the area show that the average weekly consumption of fish is 482 g and the weekly intake of THg was  $2.72 \mu\text{g kg}^{-1}$  bw. The estimated weekly intake of mercury is greater than the value established for the provisional tolerable by OMS/FAO. Even though the THg levels in fish do not exceed Cuban guideline for fish consumption, the consumption frequency exceeds the recommended levels for safe consumption by vulnerable people. Therefore, we suggest that precautionary guidelines should be established on fish consumption from the river until more data are available on Hg contamination.

#### **Development of dietary methylmercury thresholds for wild piscivorous fish**

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Wild piscivorous fish are primarily exposed to mercury (Hg) in the form of methylmercury (MeHg) via dietary uptake. Due to biomagnification of MeHg, long-lived piscivorous fish may be at risk for elevated dietary MeHg intake, accumulation and toxicity. However, compared to humans and piscivorous wildlife, little is known regarding the toxicological significance of environmentally realistic concentrations of MeHg in the diet. In controlled feeding studies in the laboratory, multiple toxic effects on behaviour, reproduction, growth and development, and cellular pathology have been observed at both environmentally realistic and extremely high MeHg concentration in dietary items. Altered growth, reproductive endpoints, and cellular pathology attributable to MeHg have also been observed in wild fish. In this paper, we critically review the published literature to date using rigorous screening criteria to develop threshold concentrations of dietary MeHg that are likely to be protective of wild piscivorous fish. The thresholds developed here will serve as criteria for a national level ecological risk assessment for piscivorous fish as part of the Canadian Mercury Science Assessment.

#### **Estimating sediment quality thresholds to prevent restrictions on fish consumption: Application to PCB and dioxins/furans in the Canadian Great Lakes**

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Sediment quality thresholds (SQTs) are used by a variety of agencies to assess the potential for adverse impact of sediment associated contaminants on aquatic biota, however SQTs that would result in fish concentrations below consumption advisory levels should also be considered. To illustrate how this can be addressed, we first calculate BSAFs for total-PCB

and dioxins/furans in the Canadian Great Lakes using measured sediment and fish concentrations in four fish species. Using these BSAFs and fish consumption advisory levels of Ontario Ministry of the Environment (OMOE), we derive fish consumption advisory-based SQTs (fca-SQTs) which are likely to result in fish levels that are safe to eat without restriction. The dioxin/furan fca-SQTs ranged from 6-128 pg TEQ g<sup>-1</sup> dw and were above the CCME's Threshold Effect Level (TEL) of 0.85 pg TEQ g<sup>-1</sup> dw. In contrast, the total-PCB fca-SQTs ranged from 1-60 ng g<sup>-1</sup> dw and were generally below the CCME's TEL of 34.1 ng g<sup>-1</sup> and OMOE's Lowest Effect Level of 70 ng g<sup>-1</sup>; however, they were consistent with the OMOE's No Effect Level of 10 ng g<sup>-1</sup>. This analysis provides approximate sediment concentrations necessary for reducing fish consumption advisories for each of the Canadian Great Lakes.

#### **Health status of wild fish from the St. Lawrence River (Cornwall) Area of Concern: 1. Biological effects**

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Environment Canada has initiated studies using various measures of endocrine function and overall fish health to readdress fish health issues in the Canadian Areas of Concern. The present study reports on brown bullhead (*Ictalurus nebulosus*) and yellow perch (*Perca flavescens*) collected from three locations in the St. Lawrence Area of Concern: Cornwall (exposed), Gray's Creek (downstream) and Morrisburg (reference). Fish health assessments used estimates of age structure, energy storage and energy utilization. Endocrine assessments included plasma steroid concentrations, *in vitro* steroid synthesis in female fish, plasma concentrations of vitellogenin, and thyroid status. Liver EROD activity was also measured. The gonads were collected and inspected for presence of deformities, and liver samples were collected and analyzed for the presence of tumors. Exposure to endocrine disruptors was also investigated by deployment of semi-permeable membrane devices (SPMDs), which were extracted and analyzed using sex steroid binding protein and androgen receptor assays. Preliminary results indicate significant differences between exposed and reference sites for several variables, including decreased plasma vitellogenin concentrations and *in vitro* steroid synthesis what about it in females collected at the exposed site. Those differences do not appear to be attributed to the presence of estrogenic or androgenic compounds in the water column.

#### **Meta-data analysis of impacts of municipal biosolids land application on soil microbes**

LAURSEN, A. <sup>1</sup>, MCCARTHY, L. <sup>1</sup> and BOSTAN, V. <sup>1</sup>. <sup>1</sup>Ryerson University

In recent years many municipalities have adopted advanced wastewater treatment technologies. Ironically, this has compounded another waste management challenge, disposal of residuals (sludge and biosolids). Landfilling is not a sustainable option, and incineration is extremely unpopular. Land application is seemingly the most sustainable long-term option, however this has also come under scrutiny with the popular press carrying stories that present risk characterizations rather than risk assessment. The scientific community has contributed to the dialogue primarily with chemical assessments of biosolids and run-off, and studies on fate and transport of pathogens. However, relatively little work has been done in the way of ecological risk assessment of land application of biosolids. In this study a meta-

data analysis is presented of microbial responses to municipal biosolids applied to agricultural land. Results show that total microbial biomass and respiration are positively correlated with biosolids loading per unit time. However, heterotrophic cell counts and most reported microbial enzymatic activities have no relationship with biosolids loading. The only enzymatic activity negatively impacted by biosolids loading was alkaline phosphatase activity. While biosolids were found to enhance overall microbial activity (i.e. biomass and respiration), presumably due to organic carbon subsidy, many microbial processes were negatively correlated with cumulative metals loading. Results of the analysis suggest that current biosolids regulation and management practices in Ontario are sufficient to protect the microbial community in soils. However, we will need to be mindful of potential impacts from cumulative metals loading if there is repeated application to the same soil.

### **Shifting stable isotope signatures of aquatic organisms in response to cumulative anthropogenic disturbances in a highly developed temperate watershed**

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Stable isotope analysis of aquatic organisms can be used to identify locations where aquatic ecosystems are under the influence of anthropogenic disturbances within aquatic environments. Carbon and nitrogen isotope ratios of riffle-dwelling aquatic organisms were surveyed along a 200 km section of the Grand River exposed to diverse anthropogenic activities in southern Ontario. Downstream trends in carbon and nitrogen isotopes were compared to measures of water quality (carbon dioxide, nitrate concentrations), and to nitrogen load estimates (cumulative dissolved inorganic nitrogen and observed nitrate) to determine if trends in stable isotopes can be explained by trends in select water quality parameters. Carbon isotope ratios increased with distance downstream with distinct responses to carbon inputs from flood control reservoirs but did not relate to CO<sub>2</sub> concentrations as observed in un-impacted systems. Nitrogen isotope ratios of resident organisms increased positively with nitrate concentrations (0.1-4.0 mg L<sup>-1</sup>) and loading estimates in the first 150 km of the study reach but decreased in the final 50 km although nitrate concentrations and loads remained elevated. These results show that stable isotope responses to anthropogenic activity deviate from water quality responses and appear to be more sensitive to changes in stream biogeochemical cycles. Results may be of interest to researchers looking for addition insight into the mechanisms by which anthropogenic disturbances translate into changes in ecosystem health.

### **The use of a model fish, *Fundulus heteroclitus*, to assess sublethal embryotoxicity of individual persistent halogenated hydrocarbons accumulated in American eel (*Anguilla rostrata*)**

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Since the early 1980's, the St. Lawrence River American eel (*Anguilla rostrata*) population is undergoing a dramatic decline associated with recruitment failure, possibly related to the accumulation of persistent halogenated hydrocarbons (PHHs) in eel tissues. These compounds are transferred to the eggs and could affect growth, behaviour and survival of early life stages. As a first step, the impact of individual PHHs was assessed in the embryos



of a model marine fish species, *Fundulus heteroclitus*. After exposure by microinjection to sublethal doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and two coplanar polychlorobiphenyls (PCBs 77 and 126), survival, growth, malformations, hatching success, cytochrome P4501A and larval behaviour were evaluated. These three compounds caused a dose-responsive induction of EROD activity. *F. heteroclitus* was relatively resistant to TCDD. On the other hand, the relative toxicity of PCBs 126 and 77 compared to TCDD was much greater in *F. heteroclitus* than in other species, with an estimated TEF for EROD induction close to 1 and 0.15, respectively. TCDD (0-1280 pg g<sup>-1</sup>) and PCB 126 (0-5000 pg g<sup>-1</sup>) caused a dose-responsive reduction of larval growth and prey capture ability, while these two responses were only altered with the highest PCB 77 dose (125 ng g<sup>-1</sup>). Mechanisms leading to the observed behavioural alterations are under investigation. Evaluation of temporal changes in the embryotoxicity of tissue extracts prepared from eels captured in Lake Ontario between 1988 and 2008 is also underway.

### **Fathead minnow lifecycle tests: Links to effects in wild fish**

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Predicting and assessing effects in wild fish from standard laboratory tests is difficult. Standard short fish exposure bioassays developed in the 70's and 80's work well for assessing acute toxicity and immediate effects of compounds and effluents. In Canada, fortunately, many of the effluents we regulate are no longer acutely toxic. Some compounds, effluents and discharges, however, do have long-term impacts on fish health. To assess the effects of long term exposures, we have used the fathead minnow lifecycle bioassay for several pure compounds and mixed effluents. Lifecycle tests encompass all "critical windows" of exposure; egg, larvae, developing and maturing juvenile, reproduction of adult fish, and survival of the F1 generation. Fish lifecycle exposures to the synthetic estrogen used in birth control pills, ethinyl estradiol (EE2), show thresholds of effect (on F1 egg fertilization success) in the 1 ng L<sup>-1</sup> range. This lab-derived cut-off mirrors responses to EE2 in wild minnows. Lab lifecycle exposures to municipal wastewater effluents (MWWE) show changes in secondary sex characteristics and decreased breeding, despite increased growth. Wild fish exposed to MWWE show similar effects on sex characteristics. Effects of pulp mill effluents on fish growth and reproduction in lifecycle studies also mirror effects seen in some wild fish. The lifecycle tests have the disadvantages of being expensive and lengthy. However, for mimicking effects of real environmental exposures, and for use in risk assessments, these tests provide valuable data that are difficult or impossible to obtain using shorter lab fish exposures.

### **Assessing the sublethal effects of in-river concentrations of parameters found to be contributing to cumulative effects in the Athabasca River Basin, using a fathead minnow bioassay**

SQUIRES, A. <sup>1</sup> and DUBÉ, M. <sup>1</sup>. <sup>1</sup>University of Saskatchewan

The Athabasca River, Alberta, Canada, is a glacial-fed river system with a basin covering 157 000 km<sup>2</sup> and is the longest (1538 km) unregulated river in the prairies. The basin holds significant cultural and economic importance supporting more than nine First Nation groups, and providing water to hundreds of industries. Previous research has quantified spatial (along the river continuum) and temporal (pre-development and present day) changes in water quantity and quality in the river. Changes in salinity and dissolved sulphate in particular have



changed significantly over space and time. Laboratory testing has linked concentrations of these parameters to changes in fathead minnow reproduction. Further research is required to determine whether these changes observed in the laboratory can be applied to actual in-river conditions. The objectives of this research were to 1) assess changes in fathead minnow response metrics (i.e., condition, liver and gonad size, egg production, gill histology) associated with increasing concentrations of salinity and dissolved sulphate and 2) determine whether sublethal effect thresholds established in laboratory experiments correspond to actual in-river concentrations using water from the mouth and headwaters of the Athabasca River. Three dose-response experiments (NaCl, SO<sub>4</sub> and water sampled from the Mouth of the Athabasca River) were conducted at Jasper National Park, Alberta, Canada using the THREATS experimental trailer. This research contributes to method development for watershed-scale cumulative effects assessment including development of whole river benchmarks for sublethal exposures of fish for a river of economic and cultural importance and experiencing significant development pressure.

### **Laboratory and field can be linked: The team approach to ecosystem assessment**

*NORWOOD, W. <sup>1</sup>. <sup>1</sup>Environment Canada*

In order to adequately protect and/or to remediate ecosystems, sound environmental decisions must be made. However, our ecosystem managers require information that clearly identifies impacts on a system and the cause of those impacts. In order to provide this information the following four questions, described in the Aquatic Effects Technology Evaluation program (ESG International Inc. 1999), need to be answered. 1) Are contaminants getting in the system? 2) Are contaminants bioavailable? 3) Is there a measurable response? 4) Are the contaminants causing this response? The data required to answer these questions is best provided by a team of specialists in analytical chemistry, ecology and toxicology. Examples of how macroinvertebrate ecotoxicity testing can provide such data by the use of laboratory and field assessments and the integration of chemical analyses, benthic community structure analyses, toxicity testing and bioaccumulation analyses, will be discussed.

### **Developing indicators of ecosystem health in the Great Lakes using natural microbial and planktonic communities**

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Across the globe, aquatic ecosystems have been adversely affected by multiple anthropogenic stressors including pollution, eutrophication, over-exploitation of fisheries, the establishment of exotic biota and climate change. Mitigating the impacts of such stressors requires integrated, adaptive, science-based approaches to management. Generally, there is a communication gap and lack of integration between toxicology and ecology. Experiments conducted in the laboratory are not applied to the field and vice versa. An integrated strategy including both laboratory and field based techniques are badly needed to generate data applicable to natural ecosystem conditions. Our paper will deal with some of the approaches we have adapted in conducting multi-trophic bioassays with natural microbial and planktonic communities as stressed organisms. In addition, laboratory grown cultures were also

conducted to complement the field results. Examples from our experience from selected Areas of Concern will be presented.

### **Putting "health" back into ecosystem health assessment**

*MUNKITTRICK, K. <sup>1</sup>. <sup>1</sup>University of New Brunswick*

There are many different purposes to collecting information, and trying to merge predictive and retrospective frameworks is very difficult. A major challenge is that situations where toxicity is strong show positive results across a spectrum of responses, including laboratory and field approaches. Regardless of the assessment purpose, objectives, approach, design and analysis, indicators have to be practical, simple, and consistent, with a low type II error rate. Laboratory approaches sacrifice ecological relevance, while field approaches have to make compromises in terms of conflicting requirements for protection, detection, reversibility, causality and ecological relevance. Ecosystem health assessment has largely followed a human health medical paradigm, where reference collections seek to define the level of natural variability in terms of spatial and temporal fluctuations that can mask impacts. Historically, definitions of "normal" levels of responses are used to detect deviations from normal and the absence of "health". There is perhaps a more useful paradigm, where the ability of organisms to integrate responses can be used holistically to evaluate performance. Under this effects-based paradigm, study designs seek out natural variability, and a system self-defines its level of health, thresholds of responses, and triggers to inform management decisions. Relationships also translate ecological information into currencies relevant for land use planning, natural resource management and impact mitigation. It represent a simpler site-specific approach that requires a commitment to baseline monitoring, consistency, and commitment to long term planning that is usually absent in current situations.

## ***Oil Sands Development & Production***

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### **Indicators of early successional trends in environmental condition and community function in constructed wetlands of the Athabasca oil sands region**

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Environmental condition criteria vary in importance according to evaluators' perspective. Biodiversity measures emphasize rare taxa. Productivity & functional indicators emphasize processes, and are dominated by common taxa. Successional indicators reflect relative composition of taxa. All measures require suitable reference systems, which are themselves regulated by interannual environmental variation. We contrasted biological, ecotoxicological & carbon dynamic aspects of sixteen 5-30 y old wetlands in post-mining Athabasca oil sands landscapes, varying in age, reclamation materials (oil sands process tailings/water), and augmentation with stockpiled surface materials (peat/mineral soil). We surveyed composition, determined carbon fluxes, and measured standing stocks of hydrocarbons, organic substrate, bacterioplankton, phytoplankton, biofilm, macrophytes, litter, zoobenthos, and aquatic insect emergence. Carbon pathways, fluxes and budgets were

determined from gas fluxes, microbial, plant, zoobenthic, amphibian, and tree swallow nestling production, and stable isotope signatures. Coarse taxon richness in reference wetlands reached an asymptote in 5-7 y. Overall richness, composition & emergent plant cover of oil-sands-affected wetlands converged slowly (15-20 y) with reference wetland patterns. Peat addition accelerated development of emergent but not submergent plant cover & associated biota. Water chemistry was more important than sediment in regulating submergent biological properties. Ultimately, residual salinity may be the most important regulator of community composition. Overall, successional trends are slower than those observed in more temperate biomes.

### **Using gas flux to estimate biological and chemical sediment oxygen demand in oil sands-affected wetlands**

GARDNER COSTA, J. <sup>1</sup>, SLAMA, C. <sup>1</sup> and CIBOROWSKI, J. <sup>1</sup>. <sup>1</sup>University of Windsor

We tested whether carbon loss in the form of sediment-associated microbial respiration differed between oil sands process-affected (OSPM) and reference wetlands. The constituents of OSPM wetlands (high salinity, conductivity and naphthenic acid concentrations) were expected to strain microbial communities and alter gas (methane and carbon dioxide) flux rates as well as sediment oxygen consumption compared to fresher, reference wetland sites. Studying 4 OSPM and 4 reference wetlands over the summers of 2009 and 2010, we found OSPM wetlands released less gas (~10 x) (methane) than reference wetlands. In 2009 we measured sediment oxygen demands (SOD) and used gas flux estimates of carbon dioxide to estimate biologically sediment oxygen consumption (BSOD). Chemical sediment oxygen demand (CSOD) was estimated by subtracting BSOD from total SOD. We found SOD rates were 2 x higher in OSPM wetlands than reference; CSOD was greater than biologically consumed oxygen for both wetland classes (~90% of total SOD). OSPM wetlands released less than reference wetlands, suggesting less microbial activity in OSPM wetlands; however, more oxygen is consumed (likely chemical oxidation) in OSPM than in reference wetlands. Carbon accrual is a necessary condition to reclaiming boreal wetlands in the Alberta Athabasca region. Less microbial activity may promote carbon accumulation (reduced respiration = less carbon loss) within OSPM wetlands but high chemical oxygen consumption may limit benthos respiration and reduce organic inputs into a wetland's sediment organic layer.

### **Response of *Chlamydomonas reinhardtii* to naphthenic acid exposure**

GOFF, K. <sup>1</sup>, HEADLEY, J. <sup>2</sup> and WILSON, K. <sup>1</sup>. <sup>1</sup>University of Saskatchewan, <sup>2</sup>Environment Canada

Oil sands process water (OSPW) is a highly toxic mixture of sediments, bitumen, ions, and organic and inorganic compounds. Naphthenic acids (NAs) are organic compounds found to be agents of primary toxicity across kingdoms, and are a contaminant class of particular concern. Bioremediation techniques have the potential to mitigate toxicity of OSPW in general, and NAs in particular. Studies of the biodegradation of NAs have focused largely on the role of bacteria, fungi, and emergent macrophytes. However, algae can potentially play an integral role in such programs, through direct degradation, biosequestration, or photosynthetic aeration of waters to promote other biological reactions. Of the various algal species identified in OSPW, *Chlamydomonas frigida* is of particular interest; however no cultures are currently available. It was therefore decided to begin analysis of potential algal

bioremediation of OSPW using *C. reinhardtii*, a well-characterized model organism. Cultures of *C. reinhardtii* were grown heterotrophically in nutrient media spiked with a dilution series of NAs. Culture densities were measured over time and used to compile growth curves, changes in rate of growth, and survivability. The concentration of NAs in solution in relation to growth rate and culture density was determined using negative ion electrospray mass spectrometry. The tolerance of *C. reinhardtii* to NAs and a proposed mechanism for this tolerance will be discussed.

### **Productivity of Chironomid larvae exposed to oil sands process water: In situ vs. lab bioassay results**

KENNEDY, K. <sup>1</sup> and CIBOROWSKI, J. <sup>1</sup>. <sup>1</sup>University of Windsor

Productivity of wetland biota is one of the key criteria that the Alberta government uses to determine if land leased to oil sands mining companies is restored. Oil sands process water (OSPW) is enriched to contain potentially toxic concentrations of salts and naphthenic acids, compromising its value in wetland reclamation procedures. We determined how Chironomid productivity is affected by the water from oil sands process material (OSPM)-affected wetlands in 10-d in situ and laboratory bioassays that used water from three OSPM-affected wetlands compared to or mixed with water from three reference wetlands of similar age. Parallel studies were conducted with *Chironomus riparius* lab-cultured larvae and *Chironomus* sp larvae cultured from egg masses collected from an OSPW-affected wetland. In situ, Chironomids were housed in 10 cm (ID) 30 cm tall cylinders with fine-mesh netting windows that allowed water exchange and contact with the sediment. Production of Chironomids emerging from study wetlands was estimated by collecting exuvia in floating hoop traps and adults alighting on sticky traps. Preliminary results suggest that native and lab cultured Chironomids are not equally sensitive to OSPW.

### **Creating wetlands in post-mined landscape: the response of vegetation**

ROY, M. <sup>1</sup> and FOOTE, L. <sup>1</sup>. <sup>1</sup>University of Alberta

One mandate of the oil sands industry is to return the post-mined landscape of Alberta to an ecozone equivalent of the Boreal Plain wetland. This is complicated because the oil sands industry must at the same time dispose of soil and water from processing bitumen, and wetlands are often used to receive tailings or soluble run-off from tailings. Our study will provide information on wetland vegetation community composition under a range of created and natural environmental conditions to improve wetland reclamation practices by 1) identifying environmental variables influencing vegetation composition within natural and created wetlands, and 2) investigating options for disposal of processed material in created wetlands that are compatible with vegetation and functional diversity. In 36 sample wetlands (20 natural and 16 created), we used a vegetation transect method coupled with plot assessments to characterize plant communities. Multivariate Regression Tree (MRT) and Canonical Correspondence Analysis (CCA) allowed us to examine relationships between water chemistry, depth, substrate types, and wetland age in relation to plant community composition. The analyses suggest that wetland vegetation composition is significantly influenced by the substrate type, water depth, water DO, water salinity and water pH. The results show that the addition of processed material to created wetlands lowered species diversity and favoured the dominance of different species than those found in natural



wetlands. Furthermore, the absence of key species in created wetlands raises questions about fragmentation effects whereby isolation of created wetlands in the post-mined landscape reduces the ability of native plant species to pioneer such newly created habitats in meaningful time, thereby leading to a need for planning changes or vegetation management.

### **Assessing the effects of the oil sands mining industry on wetland plant physiology performance**

MOLLARD, F. <sup>1</sup>, ROY, M. <sup>1</sup>, FREDERICK, K. <sup>1</sup> and FOOTE, L. <sup>1</sup>. <sup>1</sup>University of Alberta

Created wetlands of the Fort McMurray post-mined landscape have been amended with oil sands processed substrates (CT, MFT) and water (OSPW). Plant species found in these wetlands appear to tolerate the salinity and toxicity (Naphthenic Acids, Heavy Metals) of the processed materials. They offer an opportunity to identify plant mechanisms responsible for their ability to tolerate polluted wetlands. We propose that plants growing in wetlands amended with oil sands processed material may demonstrate measurable biological stress symptoms even though their physical appearance and performance seems to remain unaffected. We studied *Carex aquatilis*, an abundant wetland plant species in the Fort McMurray region in five natural (reference), and ten affected wetlands. We measured plant physiological processes: photosynthesis, transpiration rates, stomatal conductance, and leaf fluorescence. Our results show that *C. aquatilis* critical physiological processes, including carbon assimilation and water economy, are not significantly different between treatments. On the other hand, processes related to leaf light use efficiency (Fv/Fm, Performance Index) were significantly higher in plants growing in oil-sands-affected wetlands. Results suggest that processed materials can positively affect some plant photosynthetic stages but they are fully compensated and have a non-significant impact in the entire carbon assimilation process. Our results are coherent with previous research that suggests that processed materials slightly and positively enhance *Typha latifolia* performance.

### **Development of a bioassay using walleye (*Sander vitreus*) to assess the toxicity of oil sands sediments**

TURCOTTE, D. <sup>1</sup>, YUAN, H. <sup>1</sup>, RAINE, J. <sup>2</sup>, TUMBER, V. <sup>1</sup> and PARROTT, J. <sup>1</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>University of Saskatchewan

Walleye (*Sander vitreus*) are a commercially important fish species native to Canada and the northern United States. Walleye inhabit the Athabasca River, which flows through the Athabasca oil sands, and are exposed to natural sources of bitumen eroding from the McMurray formation. Walleye are very difficult to culture in the laboratory and no methods are available to test the effects of toxicants to this species of fish. Therefore, there is no information available related to the development of walleye exposed to the bitumen present in the Athabasca River. The current study describes the design and initial implementation of a daily-renewal bioassay to assess the potential effects of sediments from the Athabasca oil sands area on walleye development. Eggs were collected and fertilized with milt from spawning wild walleye captured from Lake Diefenbaker, SK. The fertilized eggs were exposed to treatments containing different concentrations of sediments or culture water only (negative controls) until complete yolk absorption of control fish, approximately 15 days post-hatch. Brine shrimp were fed to the walleye embryos daily after hatching for the remainder of the experiment. Developing fish were examined for morphological deformities (edema, bent



spine, and haemorrhage), survival, hatching success, and changes in weight and length between treatments. Whenever possible, organics concentrations in fish tissues and water were measured. A comparison with fathead minnows and northern pikes exposed to the same sediments will also be done to determine the relative sensitivity of the three species. The results of this study will provide much-needed information on the effects of sediments from the Athabasca oil sands area on fish development and survival.

### **Carbon dynamics in wetland restoration**

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Boreal wetlands play an important role in global carbon balance but their ecosystem function is threatened by direct and regional anthropogenic disturbance and climate change. Oil sands mining in boreal Canada leaves large areas in need of reclamation and generates considerable quantities of extraction-process-affected materials. It is important to know whether reclaimed wetlands are restored to equivalent ecosystem function. We approached this by assessing carbon flows and food web structure in oil-sands-affected wetlands to evaluate whether a prescribed reclamation strategy, topsoil amendment, accelerates reclaimed wetland development leading to self-sustaining peatlands. We determined carbon fluxes and measured compartment standing stocks for residual hydrocarbons, organic substrate, bacterioplankton, phytoplankton, biofilm, macrophytes, detritus, zoobenthos and aquatic-terrestrial exports (i.e. aquatic insect emergence). Most biotic compartments differed between oil-sands-affected and reference wetlands. This difference declined with age since construction but was still detectable in older (15-20 y) wetlands. Macroinvertebrate trophic diversity was lower in oil-sands-affected wetlands. Peat amendment appeared to speed convergence for some compartments but not others. We discuss results in the context of restoration of ecosystem function and optimization of reclamation strategies.

### **Novel wetland reclamation design: The Suncor Pilot Fen and Wapisiw Wetland**

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Wetlands covered about half of Suncor Energy Inc. (Suncor)'s lease prior to disturbance by oil sands mining. Fens were the dominant wetland type. Significant efforts have focused on recreating wetland ecosystems, mainly marshes, in Suncor's reclaimed landscape since the 1980s. Despite their prevalence, however, little effort has focused on recreating fen ecosystems until recently. The Suncor Pilot Fen will be one of the first ever constructed in the world. Fens are beneficial to reclaimed landscape because they support a variety of aquatic plant communities and wildlife and have large carbon and water storage capacities. The Suncor Pilot Fen program examines the feasibility of constructing a fen watershed on a former oil sands mine using tailings. The program includes: research—determining fen plant species tolerant to tailings and saline conditions—and site investigation, design, construction

and monitoring. Another innovative program, the Wapisiw Wetland Program, will evaluate whether new habitat features will increase floral and faunal diversity and abundance in a newly constructed marsh built on a former tailings pond, Wapisiw Lookout (Pond 1) – the first reclaimed oil sands tailings pond. Some of the new aquatic habitat features include: Canadian toad (*Bufo hemiophrys*) hibernacula, bird and bat nesting boxes, riparian revegetation techniques, and floating ratroot (*Acorus americanus*) islands. An overview of these two innovative wetland reclamation programs will be provided. Knowledge from these programs will help shape future reclamation efforts of Suncor and other oil sands producers striving to return sustainable ecosystems that existed before disturbance to mined landscapes.

### **Growth of *Chironomus dilutus* larvae exposed to ozone-treated and untreated oil sands process water (PO)**

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Conventional extraction processes utilized at the Alberta oil sands require extensive freshwater and produce vast quantities of oil sands process water (OSPW) which must be stored on-site. Pressure to remediate OSPW and return it to natural systems has encouraged investigation of different treatment methods that may eliminate downstream toxicity in future release scenarios. The acute toxicity of OSPW has been mainly attributed to naphthenic acids, so these are considered the most important target fractions for treatment. Ozonation has shown potential for reducing OSPW toxicity, but effects of ozonation on aquatic invertebrates are unknown. In this study, the effects of exposure to untreated and ozonated OSPW were investigated in *Chironomus dilutus* larvae. OSPW was treated with either a 50 or 80 mg O<sub>3</sub> L<sup>-1</sup> dose of ozonation, and both ozonation levels were examined for effects on *C. dilutus* survival and growth. Following a 10-day exposure, there were no significant effects (relative to freshwater controls) on survival of larvae exposed to either ozone-treated or untreated OSPW. OSPW-exposed larvae were 64-77% smaller than their respective controls (p<0.05), but the mean wet mass of organisms exposed to 50 mg O<sub>3</sub> L<sup>-1</sup> ozonated OSPW was not significantly different from that of the controls (p=0.486). Larvae exposed to 80 mg L<sup>-1</sup> ozonated OSPW were only 40% smaller than the freshwater controls (p<0.001), with mean wet mass that was also significantly larger than the untreated OSPW (p=0.001). These results suggest that ozonation may be a viable treatment method to reduce toxicity of OSPW to benthic invertebrates.

### **Minimal health impacts but detectable tissue residues after exposure of northern leopard frogs (*Lithobates pipiens*) to commercial naphthenic acids (PO)**

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Naphthenic acids (NAs) have been implicated as one of the main causes of the toxicity related to oil sands process-affected materials (OSPM). However, previous studies suggest that NAs may not be the toxic component of OSPM. This study investigated the toxicity of commercial (Refined Merichem) NAs to native amphibians (northern leopard frogs) concurrently exposed to saline conditions comparable to those of reclaimed wetlands on the oil sands. Frogs were kept in NAs solutions (0, 20, or 40 mg L<sup>-1</sup>) for 28 days. These exposures

resulted in proportional NAs concentrations in extracts of frog muscle tissue, estimated by GC-MS analyses. Biological assays determined if the increasing exposure concentrations of NAs, and subsequently increased tissue NAs levels, caused a proportional compromise in the health of test animals. Investigations of innate immune function, thyroid hormones, and hepatic detoxification enzyme induction did not differ in response to increased tissue concentrations of NAs. Our results demonstrate that commercial NAs are absorbed and deposited in muscle tissue, and that NAs may play a small role, if any, in the toxicity of OSPM to frogs.

#### **Changes in algal stable isotopes following nutrient and peat amendments in oil sands aquatic reclamation (PO)**

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The extraction of bitumen from oil sands in Alberta, Canada, generates a large amount of processed material that must be reclaimed. Processed water and solids (PW/S) contain elevated levels of naturally occurring compounds including naphthenic acids (NAs) and polycyclic aromatic compounds (PACs). Constituents associated with PW/S, including organic carbon (NAs and PACs) and nitrogen (mainly as process-added  $\text{NH}_4^+$ ) may provide nutrient sources as aquatic reclamation sites develop into viable ecosystems. The objective of the current study was to evaluate modifying factors that may influence the stable carbon and nitrogen isotope values of primary production in oil sands aquatic reclamation. Using both field-based microcosm (water/substrate) studies and laboratory (water soluble fractions only) studies, changes in the growth and stable isotope values of phytoplankton, periphyton and/or filamentous algae along gradients of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), nitrogen and phosphorus were evaluated. The studies utilized differing types of reclamation substrates (sand; mature fine tailings (MFT; 50%) + sand (50%); peat + MFT/sand) and process water. Results showed different levels of growth depending on both the water and substrate type. Generally, periphyton from oil sands reclamation sites (DP and MP) were more  $^{15}\text{N}$  enriched than the reference site (SWSD). Periphyton from MP was more  $^{13}\text{C}$  enriched than periphyton from SWSD whereas DP periphyton was more  $^{13}\text{C}$  depleted than the reference site. Previous research suggests that oil sands reclamation sites may be more  $^{13}\text{C}$  depleted but this is not the case for MP. This suggests there are other factors (i.e., nutrients) influencing carbon isotopes.

#### **Stable carbon and nitrogen isotopes of tree swallows influenced by oil sands aquatic reclamation (PO)**

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Tree swallows (*Tachycineta bicolor*), have been studied as upper trophic-level sentinels to assess reclamation strategies on the Alberta oil sands. Wetland reclamation incorporates tailings water and solids (from the extraction of bitumen from sand) into functioning aquatic ecosystems. Naphthenic acids (NAs) and polycyclic aromatic compounds (PACs) in tailing/reclamation material are constituents of concern for growth rate, reproductive function and immune function in tree swallows. Previous research indicated no differences in these endpoints from tailings additions; however, increased detoxification enzyme (EROD)

activity indicated exposure to xenobiotics from these tailings-based sites. Stable isotope analyses are studied here to further define dietary exposure of tree swallows to oil sands constituents. Previous studies have shown trends of  $^{13}\text{C}$  depletion and  $^{15}\text{N}$  enrichment in benthic invertebrates from reclaimed wetlands on the oil sands. Because the majority (84%) of dietary items consumed by tree swallow nestlings is aquatic, the isotope signatures in tree swallows should provide evidence of exposure to oil sands constituents. Here, tree swallow feather and muscle tissues were examined to determine if stable isotopes could be used to distinguish dietary contributions from oil sands reclamation sites vs. reference sites containing no tailings materials.

#### **Toxicity of Athabasca River and oil sands sediments to larval fish (PO)**

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Oil sands tailings pond sediments and sediments from the Athabasca River were assessed for embryo-larval toxicity in fathead minnows and walleye. No effects were seen in fathead minnow eggs and larvae exposed for 20 days to 5 sediments from Athabasca River at concentrations up to  $25 \text{ g L}^{-1}$ . Two of three tailings pond sediments were toxic to fathead minnows at concentrations as low as  $0.2$  to  $1 \text{ g wet wt L}^{-1}$ . All three tailings pond sediments reduced growth (lengths) of larvae. Nine of 18 polycyclic aromatic hydrocarbons (PAHs) measured in tailing pond sediments were above the CCME Freshwater Sediment Quality Guidelines, with phenanthrene being the highest at  $19 \mu\text{g g}^{-1}$  dry wt. Total PAHs were up to  $56 \mu\text{g g}^{-1}$  dry wt. Total naphthenic acids in tailings pond sediment were up to  $16 \mu\text{g g}^{-1}$ . River sediment had up to  $2.6 \mu\text{g g}^{-1}$  naphthenic acids and up to  $0.25 \mu\text{g g}^{-1}$  total PAHs. Results are preliminary, and we cannot at this time say what is causing the observed toxicity in fathead minnow larvae. Assessment is continuing with exposure of walleye eggs and larvae, to compare their relative sensitivity to these sediments, and to assess the potential causative compounds.

#### **Compromised development and survival in amphibians in reclaimed wetlands' water containing oil sands process-affected material (PO)**

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Government regulations require that mine sites, at closure, be ecologically sustainable, supporting endemic flora and fauna. One reclamation strategy is creating wetlands to age and detoxify oil sands process-affected materials (OSPM). To address a gap in knowledge, we investigated whether amphibians indigenous to this boreal forest ecosystem can complete their lifecycle in water from the reclaimed wetlands. Wood frog (*Lithobates sylvaticus*) eggs were collected from a natural pond 60 km south of the oil sands mining activity. Tadpoles were individually housed and reared in 1 of 6 water treatments refreshed every two days: aged tap water (reference 1), water from a natural wetland (reference 2), or water from 4 research wetlands on Syncrude and Suncor lease sites (120 tadpoles / treatment). There were no significant differences in growth, development, or survival rates between the aged tap water and reference wetland water. These two reference groups had the fastest growth, development, and highest survival rates. In contrast, there were significant differences among the 4 treatment groups from Suncor & Syncrude reclamation sites. Survival was high in 3 of the water treatments from Syncrude/Suncor sites, but development rates were significantly reduced. Delayed development in tadpoles poses a serious threat to population



stability in OSMP-containing wetlands because tadpoles that fail to metamorphose prior to freeze-up do not survive.

### **Development of a bioassay to assess the toxicity of oil sands sediments to pike (*Esox lucius*) (PO)**

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Pike (*Esox lucius*) are a commercially important fish species that are native to the northern hemisphere. In Alberta (Canada), pike inhabit the Athabasca River, which flows through the Athabasca oil sands, and are exposed to natural sources of bitumen eroding from the McMurray formation. Pike are easily cultured in laboratory environment; however, no methods have been developed to assess the toxicity of oil sands to this fish species. Therefore, there is no information available related to the development of pike exposed to the bitumen present in the water. The current study describes the design and initial implementation of a daily-renewal bioassay to assess the potential effects of sediments from the Athabasca oil sands area on pike development. Eggs were collected and fertilized with milt from spawning wild pike captured from Lake Diefenbaker, SK. The fertilized eggs were exposed to treatments containing different concentrations of sediments or culture water only (negative controls) until complete yolk absorption of control fish, approximately 15 days post-hatch. Brine shrimp were fed to the walleye embryos daily after hatching for the remainder of the experiment. Developing fish were examined for morphological deformities, survival, hatching success, and changes in weight and length between treatments. Preliminary results suggest that pike are less sensitive than walleye and fathead minnow to the toxicity of oil sands sediments.

### **The chemical and biological evolution of mature fine tailings in oil sands end-pit lakes (PO)**

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Sediment Oxygen Demand (SOD) is a major contributor to oxygen depletion in wetlands. Biochemical processes occurring at the sediment-water interface can influence wetland ecosystem function and sustainability in accordance with sediment composition. The biochemical reactions associated with natural sediment can be altered by the presence of oil sands processed material (OSPM), which can affect SOD and, by limiting oxygen concentrations in the overlying water, affect ecosystem viability. Establishing the biotic and abiotic controls of SOD is imperative in assessing current wetland reclamation designs' effectiveness at restoring productivity to those observed during pre-mining conditions. In this laboratory microcosm laboratory, the REDOX chemistry of fresh tailings sediment (MFT) was measured to investigate chemical and biological influences and to study the role of developing microbial communities as new MFT ages. Changes in the principal chemical, physical and biological populations of the MFT were assessed in both aerobic and anaerobic microcosms using a combination of microelectrode arrays and DNA profiling at the tailings water interface. In addition to preliminary findings, we present a novel bench-scale technique to characterize tailings and their impact on sediment oxygen demand (SOD) for future end-pit lake model behaviour. We also discuss how our laboratory-based microcosm results can be validated under field conditions.



## **Molecular identification of a yellow perch viral disease associated with exposure to oil sands process affected waters (PO)**

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Oil sands processing produces large volumes of tailings and process-affected water which must be safely incorporated into the terrestrial and aquatic landscape. Yellow perch were stocked into experimental ponds, Demonstration Pond and South Bison Pond, during the periods 1995-1997 and 2008-2010. Demonstration Pond consists of mature fine tailings capped with natural surface water; South Bison Pond was formed in an area surrounded by overburden and/or lean oil sands. Disease surveys were conducted at these locations and at three reference locations: Mildred, Sucker, and Kimowin Lakes. External white nodular lesions, characteristic of lymphocystis disease, were observed on perch in all but Kimowin Lake. DNA extraction and PCR with genotype generic major capsid protein gene primers were performed to confirm the identity of the virus. Sequencing of PCR results confirmed the presence of lymphocystis disease virus in perch and suggested that the viral genotype isolated in this study may be different from any previously isolated. There was an increasing incidence of the disease in Demonstration Pond and a decreasing incidence in the South Bison Pond over the duration of the time periods. The severity of this disease is proportional to the incidence. These changes in incidence are positively correlated with changes in naphthenic acid concentration.

## ***Cancer in Bivalves***

### **Leukemia in molluscs: A model of environmental carcinogenesis**

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Bivalves are particularly useful as aquatic monitors in Atlantic Canada and in British Columbia because, in addition to other attributes, they develop pollution-associated tumors. Based upon pioneering work of Yevich and Farley, our laboratory developed a monoclonal antibody (MAB) to the tumor cell of *Mya arenaria*. As a result, we redefined the tumor as a leukemia with many similarities to human monocytic leukemias. Our lab found that: 1) the MAB identified a 250KD glycosylated protein; 2) specific genes are preferentially expressed (p53/63/73) depending upon whether or not a cell is normal or fully leukemic; 3) there is a definite link between certain types of pollution and leukemia. Where is the research headed? In terms of molecular markers, how p53 gene family members are expressed and internally regulated is being defined. This is extremely important because the tumor suppressor family (p53) is also expressed in humans. The techniques to indefinitely grow clam leukemia cells in vitro, pioneered by Walker et al., now provide an invaluable tool for defining mechanisms of gene modulation using chemotherapeutic agents. Our team, headed by James Sherry, is examining how exposure to agrochemicals in PEI influences leukemia development and gene expression. As you will hear, the molluscan model is providing critical data for cell and molecular biologists, environmental assessors, modelers, and evolutionary virologists.

## Haemic neoplasia in Prince Edward Island soft-shell clam (*Mya arenaria*): Is there a link to pesticide use in upstream potato fields?

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Intensive farming of potatoes on Prince Edward Island (PEI) relies on the repeated and widespread application of pesticides – particularly during blight conditions. Our hypothesis is that pesticides or their degradation products might accumulate in the depositional zone of PEI riverine estuaries where they could impact benthic health. *Mya arenaria* (the soft shell clam or steamer) is a vital benthic component of PEI's estuarine ecosystems. As a filter feeder *M. arenaria* could be vulnerable to depositional contaminants. *M. arenaria* is susceptible to haemic neoplasia (HN), more commonly known as "leukemia". HN has a complex aetiology; there are, however, reported associations with environmental stressors. In May 2009, we established that HN rates were generally higher in PEI estuaries located downstream of high-intensity potato farming (Dunk and Wilmot watersheds) than in estuaries downstream of low-moderate intensity areas (a minimum of 120 clams were sampled at each of six sites for a total of 1189 clams). We used the fluorescently labelled MAB-1E10 technique to confirm the microscopic diagnosis of HN. In late October 2009, we confirmed our earlier data that HN rates were elevated in the Dunk and Wilmot estuaries compared to reference (Souris River) rates (137-200 clams sampled per site). Moreover, HN rates declined with distance from source along transects through the Dunk and Wilmot estuaries. Remarkably, no HN was detected in *Mytilus edulis* from the Dunk/Wilmot estuaries (120 mussels per site). Select immune, molecular, and protein expression endpoints could differentiate between groups of HN and normal *Mya*. These results have broad implications both for the aquatic benthic ecosystem and for human health. Parts of the mechanisms that underlie neoplasia in bivalves have been identified, are associated with highly conserved genes, and are similar to known cancer mechanisms in humans.

## Alterations in immune function of *Mya arenaria* from pesticide-impacted estuaries in Prince Edward Island

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This study examined immune function in *Mya arenaria* from a reference estuary (Souris) and two estuaries located downstream of high-intensity potato farming in PEI (Dunk and Wilmot). The hemolymph of 20 clams from each site was visually screened for haemic neoplasia (leukemia) prior to assessing phagocytotic activity with fluorescently labelled bacteria. Although overall there was no significant difference in phagocytosis among *Mya* collected from the three study sites, when clams were grouped according to their stage of leukemia, there were significant differences in their ability to phagocytose bacteria. Clams classified as Moderate Leukemic (20-50% leukemic cells) and Heavy Leukemic (>50% leukemic cells) exhibited significantly ( $p < 0.05$ ) less phagocytosis activity than Normal clams (no leukemic cells). An analysis of stained hemocytes (200 cells clam<sup>-1</sup>) revealed that granulocytes (those responsible for phagocytosis) account for a greater proportion of the total hemocytes in clams from impacted sites (Dunk, 16.4% (SE 2.7), Wilmot 22.0% (SE 3.3)) than those from the reference site (Souris 7.4% (SE 2.0)), suggesting compensatory stimulation in pesticide-

exposed clams. Also, when grouped according to leukemic stage, clams classified as Moderate and Heavy Leukemic had significantly ( $p < 0.001$ ) more granulocytes (26%) than normal clams (10%). Significant differences were also observed in cytoplasm to nucleus diameter ratios between pesticide impacted and reference clams. These results illustrate the subtle changes that occur when *Mya arenaria* are chronically exposed to pesticides and reveal the transition in immune function that occurs from healthy to leukemic clams.

#### Potential underlying molecular mechanisms in haemic neoplasia in bivalves

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Haemic neoplasia ("leukemia") in bivalves has been suggested for use as an environmental monitoring endpoint because environmental factors are implicated in the etiology, but many questions regarding molecular mechanisms that underlie this disease remain to be answered. This talk will provide an overview of studies exploring molecular mechanisms, focusing on the p53 tumor suppressor pathway in clams (*Mya arenaria*) and mussels (*Mytilus* sp.). It has been shown that members of the p53 tumor suppressor gene family have higher levels of protein expression in neoplastic cells. Sequencing of full-length cDNA of p53 gene family members revealed variants of different length at the 5'- translated and 3'- un-translated cDNA ends. Which copy is transcribed when and in which tissues in the bivalves remain unanswered questions. Activity of the p53 protein can be down-regulated by different mechanisms, such as Mdm-dependent ubiquitination or mortalin-dependent translocation to the cytoplasm. We have discovered that a bivalve Mdm-like protein binds p53, but the function has remained elusive. Mortalin on the other hand prevents p53 from being translocated to the cell nucleus, where it could otherwise be active as a tumor-suppressing transcription factor. Gene expression and mutation have been studied by us in mussels *M. trossulus* and *M. edulis*. We found that a combination of expression levels of several genes of the p53 pathway is statistically predictive of whether an animal has haemic neoplasia or is healthy. Interestingly, mRNA and protein levels of different p53 family members may be reciprocal. Single nucleotide polymorphisms in the p53 gene also show associations between neoplastic and healthy phenotypes. The elucidation of molecular mechanisms is an important step in the development of reliable and credible biomarkers.

#### Genetic biomarkers for haemic neoplasia in *Mytilus* spp.

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Two *Mytilus* species, *M. edulis* and *M. trossulus*, are commonly used for environmental monitoring. Both species develop leukemia, which is used as one of the endpoints. *M. trossulus* has a much higher prevalence of leukemia than *M. edulis*. Here we discuss three methods of leukemia detection in both species: visual assessment of neoplastic cells in haemolymph, analysis of polyploidy in haemocytes using flow cytometry, and genotyping—i.e., detection of single nucleotide polymorphisms (SNPs). Visual assessment is the most traditional and widely accepted method. It is, however, time consuming and requires highly trained personnel. Polyploidy analysis is more time efficient and does not depend on personal judgment. Its results are in a satisfactory agreement with the visual leukemia assessment. We found SNPs in *M. trossulus* p53 coding region associated with leukemia. We proposed to use

these SNPs as a marker for disease detection. Analysis of SNPs in *M. edulis* p53 coding region revealed SNPs associated with leukemia as well, although the positions of the SNPs for the two species are not the same. Based on the SNPs analysis, we suggested hybridisation of *M. edulis* with *M. trossulus*. The latter was confirmed by restriction fragment analysis. According to our preliminary data, hybrids are more prone to become leukemic than *M. edulis*.

### **Transcriptional and non-transcriptional induction of apoptosis by wild-type p53 following defeat of mortalin-based cytoplasmic sequestration in cancerous clam hemocytes**

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p53 is a transcription factor with many target genes involved in cell senescence, p53 down-regulation, DNA editing/repair, and apoptosis. In most cancers, p53 is mutated in the DNA binding domain, and deficiencies in all four transcriptional outcomes result. The apoptotic function is retained when mutant p53 lacking the transcriptional activation domain is employed. Efforts to account for this result reveal an additional non-transcriptional function for wild-type p53 that initiates a mitochondrial apoptotic pathway induced by significant stress (e.g., chemical). Here we use a naturally occurring, fatal cancer of soft-shell clam hemocytes to investigate cytoplasmic sequestration (CS) of wt p53 that abrogates both transcriptional and non-transcriptional apoptotic outcomes for wt p53 in cancerous clam hemocytes (CCH). The molecular mechanism that accounts for CS of wt p53 in CCH involves over-expression of the mitochondrial Hsp70 protein, mortalin, which tethers wt p53 protein in the cytoplasm. We have used the dye MKT-077 (that also binds to mortalin) to overcome cytoplasmic tethering of wt p53 in CCH. Treatment of CCH with 3.5  $\mu$ M MKT results in translocation of wt p53 protein to the nucleus and subsequent p53-dependent apoptosis. Pre-treatment of CCH with the nuclear pore blocker wheat germ agglutinin followed by MKT results in translocation of wt p53 to the mitochondria followed by apoptosis. We suggest that mortalin-based cytoplasmic sequestration of wt p53 in CCH can be overcome using MKT by both transcriptional- and non-transcriptional- induced apoptosis of CCH. The latter of these two mechanisms is of high significance in situations where chemical pollution induces significant stress. Two splice variants of clam mortalin are also reported. Supported by NIH and UNH Hatch to CWW.

### **The relationship between mitochondrial activity and gonad lipid stores in cold- and warm-water-adapted clam populations: The disruptive effect of pollution-related stress**

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The purpose of this study was to examine the relationship between mitochondrial activity and gonad lipid stores in clams exposed to anthropogenic pollution at cold- and warm-water sites. The balance between energy expenses and energy reserves was measured by mitochondrial electron transport (MET) activity and lipid content in the gonad. The activity of malate dehydrogenase (MDH) was measured as an intermediary between energy production and the production of lipids in gonadal tissues. The results revealed that intertidal clam populations at warm-water sites under no source of pollution had less heavy metal content (Ag, As, Cr, Hg and Ni), lower MDH activity and temperature-dependent MET than



clams from cold-water sites. However, MDH activity measured at 6°C was higher at the warm-water sites. Lipid peroxidation in gonadal tissues was higher in clams from the cold-water sites. The impacts of pollution differed among the study sites, clams from cold-water sites having increased MDH activity, temperature-dependent MET activity, higher lipid reserves and DNA strand breaks; clams from the warm-water sites had increased temperature-dependent MDH activity and lower gonadal lipid reserves. The enzyme affinity constant (for malate) at 25°C was lower at the polluted cold-water site. The kinetic constants ( $K_m$  and  $V_{max}$ ) did not change among clams from the cold- and warm-water sites, but the impacts of pollution in clams from the cold-water site had reduced temperature dependence in enzyme affinity and velocity ( $V_{max}$ ) towards both substrates (malate and NAD). A multiple regression analysis revealed that gonad lipid reserves were positively correlated with MDH activity and negatively correlated with its temperature-dependent activity, suggesting that increased temperature sensitivity was negatively related to gonad energy reserves. The data show that pollution increases temperature sensitivity at the MET level in clams in cold water, while temperature sensitivity in MDH activity was observed in clams from warm-water sites. A discriminant function analysis also revealed that pollution stress shows a tendency to be closer to clams adapted to warmer temperatures. In conclusion, pollution could negatively affect the temperature adaptation mechanisms in intertidal clam populations.

### *Impacts of Oil Spills and Oil Clean-up*

#### **The effects of WAF and CEWAF on EROD activity in juvenile Atlantic cod**

BURRIDGE, L.<sup>1</sup>, LYONS, M.<sup>1</sup>, WONG, D.<sup>1</sup>, LI, Z.<sup>1</sup> and LEE, K.<sup>1</sup> <sup>1</sup>Fisheries and Oceans Canada

Water-accommodated fractions (WAF) of two types of oil were generated either in a wave tank or in a baffled flask. Oil droplet sizes of the two systems were comparable as confirmed by a LISST particle size analyzer. Chemically enhanced water accommodated fractions (CEWAF) of combinations of two oils and two dispersants were prepared in a similar manner. Juvenile Atlantic cod (*Gadus morhua*) (n=20) were exposed (T=4 h) to a range of concentrations of either WAF (4.7% to 75% v/v) or CEWAF (0.5% to 64% v/v) then transferred to clean seawater. Five fish were sampled periodically over the next 68 h and liver EROD activity was measured. At the end of the 4-h exposure, EROD activity was no different from the controls. Twenty hours later EROD activity was significantly elevated compared to the controls at CEWAF concentrations above 2% v/v regardless of oil or dispersant type. EROD activity declined over the next 48 h but at T=72 h had not returned to control or pre-treatment levels. EROD in livers from cod exposed to WAF was significantly elevated after 24 h at concentrations of 12% v/v or higher, reaching a maximum of 8 picomoles/mg protein/min. EROD activity in WAF-exposed livers declined to pre-treatment levels after 72 h. These results indicate that brief, 4-h, exposure to WAF and CEWAF can induce EROD activity in livers of juvenile cod and that the elevation of enzyme activity can persist for 24-48 h.



### **Impact of water-accommodated fractions of crude oil on Atlantic cod (*Gadus morhua*) following chronic exposure**

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Mature Atlantic cod, *Gadus morhua*, were exposed to low concentrations (20-70 ppb) of water-accommodated fractions (WAFs) of polycyclic aromatic hydrocarbons (PAHs) in an ambient flow-through seawater system to determine the long-term effects on gonadal development. Some PAH-exposed cod groups were depurated afterwards for 38 to 287 days. Although mortality was rare, external lesions occurred only in the PAH-exposed groups. Gonadal development, determined by gonado-somatic index, was disrupted in both sexes and spawning and spermiation delayed in the depurated PAH-groups. These results suggest that the WAFs in the water column have an adverse effect on reproduction in Atlantic cod, a demersal species, by retarding gonadal development following chronic exposure.

### **Do oil dispersants make spilled oil more toxic to fish?**

HODSON, P. <sup>1</sup>. <sup>1</sup>Queen's University

The recent Deepwater Horizon disaster in the Gulf of Mexico was the world's largest unintentional oil spill. It was unprecedented in its duration, volume spilled, and the technology applied for control and clean-up. Among these unique features was the continuous and widespread application of oil dispersant, at the surface and at the discharge, 1500 m deep, generating public concern about dispersant toxicity and the effects of dispersion on oil toxicity. Recent USEPA reports claim little difference in acute toxicity to marine fish and invertebrate species among commonly available dispersants and between dispersed and non-dispersed Louisiana Sweet Crude. The EPA reports were technically correct: the toxicity of waterborne hydrocarbons does not vary with chemical dispersion. However, the agency did not tell the entire story, omitting any consideration of loading. Our research on the chronic toxicity of dispersed oil to fish embryos demonstrates that toxicity expressed as oil loading increases by a factor of 10 to 1000 times with dispersion, primarily because 10 to 1000 times more oil enters the water column. From a practical perspective, the risk of oil toxicity to fish increases an equivalent amount because the action of dispersant is on the exposure component of the risk equation, not on the potency of the toxic components of oil. By telling only part of the story, the USEPA seriously misled the public about the risks of dispersant use in oil clean-up.

## ***Industrial Effluent Monitoring***

### **A review of 20 years of pulp and paper mill effluent monitoring in Canada: From acute toxicity to gene expression profiling**

MARTEL, P. <sup>1</sup>. <sup>1</sup>FPInnovations

This presentation will review how chemical and biological monitoring of pulp and paper mill effluents was useful over the last 20 years in understanding and resolving some of the

aquatic toxicity issues faced by this industry. The topic is addressed from the perspective of regulatory requirements which have triggered major process changes and how these changes affected the quality of effluents in terms of the acute and chronic toxicity to aquatic organisms. Acute toxicity was mostly eliminated by biological treatment, although occasionally some cases of non-compliance have required the application of a pulp mill effluent-specific approach to identify the cause. Chronic toxicity issues, mostly revealed by field monitoring, have dominated the last 20 years and a significant part of the presentation will be focusing on the utility of life cycle and short term tests used to assess the impact of process changes and biological treatment on reproductive endpoints. These studies will also be discussed in terms of the guidance they can provide towards abating effects of effluents.

### **Challenges overcome and lessons learned from using freshwater bivalves during two Investigation of Cause (IOC) environmental effects monitoring (EEM) studies**

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Two Ontario pulp and paper mills were required to conduct Investigation of Cause (IOC) studies for their EEMs. In both cases, significant differences in fish and benthic invertebrate community endpoints were observed between reference and exposure populations during previous EEMs. However, due to confounding factors in the receiving environment (i.e. a sewage treatment plant discharge or historical sediment contamination), it could never be definitively determined whether impacts were the result of the pulp mill effluent. As such, two freshwater caged bivalve studies were designed to eliminate confounding factors. While the use marine bivalves in EEM studies is not novel, the use of caged freshwater bivalves presented new challenges, such as choosing appropriate sentinel species, sourcing test organisms, cage design, choosing organisms based on the ability or inability to assess certain EEM endpoints (i.e. growth, reproduction, condition) and integration of benthic survey results. The methods used to overcome all of these issues will be discussed. The results of the IOC EEM on the Kapuskasing River showed no statistically significant effects on the test organisms (*Sphaerium striatinum* and *Elliptio complanata*), indicating that current effluent quality is not an issue. The results of the IOC EEM in Beaverdams Creek near Thorold, ON also indicated no significant differences in reproduction between the Near-field Exposure and Reference Area using *Lasmigona compressa*. The use of *S. striatinum* for growth was not successful in Thorold due to the nature of the receiving environment and large amounts of organic debris.

### **Seasonal reproductive patterns and recommended sampling times for sentinel fish species used in environmental effects monitoring programs**

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Canada's environmental effects monitoring (EEM) program is currently in its fifth cycle of monitoring for the pulp and paper industry and second cycle of monitoring for the metal mining industry. More than 60 different sentinel fish species have been used in the EEM fish population surveys, and reproductive impacts have been identified as an issue of concern in the pulp and paper program. A review of the literature was conducted to obtain details of the reproductive biology of each fish species that has been used in EEM studies in Canada. Using

available data on seasonal changes in gonadosomatic indices, the seasonal reproductive patterns of Canadian fish species were divided into categories based on reproductive strategy and the timing of initiation of gonadal recrudescence. Recommended sampling times were developed for each reproductive pattern based on periods of temporal stability, minimum variability, and maximum value in gonadosomatic indices within a reproductive cycle. The reproductive strategy, spawning time, spawning temperature, and recommended sampling time were provided for the each sentinel fish species as well as life history characteristics including longevity, age and size at maturity, and mobility. Examination of the fish surveys using small bodied forage species from the EEM pulp and paper program revealed that approximately 72% of these studies were not conducted at the developed recommended sampling times and the magnitude of impacts may be underestimated by failing to sample at the recommended time.

#### **Selenium concentrations and loads from coal mining operations in the Elk River watershed, southeastern British Columbia: 2004-2009**

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Teck Coal Ltd. operates five coal mines in the Elk River watershed in southeastern BC. Selenium concentrations were measured monthly at 27 Receiving water stations in the Elk River and its major tributaries, and at 40 Discharge stations (point sources), from 2004 to 2009. Trends for selenium concentrations in receiving waters, and for concentrations and loads at selected discharges, were assessed. In 2004-2009, selenium concentrations were elevated well above background levels of 1 µg/L in areas downstream of mine discharges in the Fording River (a major tributary of the Elk River), Line Creek (a major tributary of the Fording River), Michel Creek (a major tributary of the Elk River below the Fording River confluence), and in the lower Elk River below its confluence with the Fording River. Concentrations increased significantly over time in the Fording and lower Elk Rivers, and in Line Creek. Relatively few (<10) major sources accounted for most of the selenium loads discharged to the Elk River and its tributaries. Concentrations increased over time at most major sources. Selenium loads increased over time at some sources, but decreased at others. In recent years (2007-2009), concentrations in the lower Elk River and at several major sources may have increased partly because flows decreased. The mines are exploring options for reducing selenium loads discharged to downstream receiving waters.

#### **Food chain model to predict westslope cutthroat trout ovary selenium concentrations from water concentrations in the Elk Valley, BC**

*ORR, P.<sup>1</sup>, WIRAMANADEN, C.<sup>1</sup>, FRANKLIN, W.<sup>2</sup> and FRASER, C.<sup>2</sup>. <sup>1</sup>Minnow Environmental Inc., <sup>2</sup>Teck Coal Limited*

Teck Coal Ltd. (Teck) operates five coal mines within the Elk River Valley of the Columbia River Basin in southeastern British Columbia. Selenium is released during weathering of mine waste rock. A number of field studies have been conducted since 1996 that measured selenium concentrations in biota and showed that tissue concentrations are higher in aquatic biota sampled in lentic compared to lotic habitats of the watershed with similar water

selenium concentrations. The available data were used to develop two food chain models that described dietary selenium accumulation in the ovaries of lotic versus lentic westslope cutthroat trout (WCT), a valued aquatic resource in the Elk River system. For each model, three trophic transfer relationships were characterized using field data: 1) water to base of the food web (epilithon, epipelon, periphyton), 2) base of the food web to benthic invertebrates, and 3) benthic invertebrates to WCT ovaries. Each model (lotic and lentic) combined the resulting equations for each trophic transfer relationship to predict WCT ovary concentrations from water concentrations. The models fit the available data remarkably well, given the confounding influence on the input data of fish movements and considering that composite benthic invertebrate sample data used in the model are only an approximation of the feeding preferences of individual fish. The models predict very small/slow increases in WCT ovary concentrations over time, based on the observed rates of increase in water selenium concentrations throughout the watershed.

**Validation of a refined short-term adult fish reproductive test with improved power for mummichog (*Fundulus heteroclitus*) to test complex effluents (PO)**

BOSKER, T. <sup>1</sup>, MUNKITTRICK, K. <sup>1</sup>, HEWITT, M. <sup>2</sup>, MELVIN, S. <sup>1</sup> and MACLATCHY, D. <sup>3</sup>.

<sup>1</sup>University of New Brunswick, <sup>2</sup>Environment Canada, <sup>3</sup>Wilfrid Laurier University

Short-term adult fish reproductive tests are widely used to assess the toxicity of chemicals and waste streams, and are currently being used as alternatives to field studies by organizations such as the OECD, Environment Canada and US EPA. However, these lab reproductive tests often have low power to detect differences in egg production among treatments, due to high variance and small sample sizes, limiting their effectiveness for informing regulatory decisions. A protocol for a fish reproductive test using mummichog (*Fundulus heteroclitus*) was refined to increase statistical power. Three studies using the original protocol were compared with three studies using the refined protocol. Tank pre-selection and enlarged sample size increased the a priori power from 11.2% to 85.7%. After exposure, average power levels were 62.0%, a more than four-fold increase compared to studies that used the original protocol (power of 15.0%). There was a high level of consistency compared to the original protocol; differences >33% in female and male gonad size and egg production could be detected among treatments. This study demonstrates that a refinement process can address shortcomings in short-term adult fish reproductive protocols with other species, creating a stronger foundation for further standardization and possible regulatory use.

### Temporal trends of organochlorines, brominated flame retardants, and mercury in eggs of Northern gannet from the Gulf of St Lawrence, 1968-2009

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Colonial waterbirds are widely used as indicators of environmental contamination and sentinels of ecosystem health. Populations of Northern gannet (*Morus bassanus*) in the Gulf of St. Lawrence were found to be highly affected by pollutants in the 1960s and 1970s. The population has been recovering ever since, and a monitoring program is in place to measure environmental contaminant levels and their association with productivity and population over time. Concentrations of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and mercury (Hg) were measured periodically in eggs of Northern gannet from the Gulf of St Lawrence from 1968 to 2009. Brominated flame retardants (BFRs) were measured in 2004 and 2009. All organochlorine compounds or groups of compounds (19) were found to decrease between 1968 and 2004, whereas 51% of the compounds (96) decreased, 47% showed non-significant trends and 2% increased (ortho-4-CB and nona-CB) from 1984 to 2004 when trends were controlled for variation of trophic level ( $\delta^{15}\text{N}$ ) over time. Population size, egg shell thickness and productivity parameters were found to be inversely related to concentrations of pollutants.

### Are mercury levels in fish-eating double-crested cormorants and Caspian terns in Lake Ontario affected by migration patterns?

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The potential of birds to act as biovector transport agents between ecosystems (e.g., through feather molting) was recently established, but to date little is known about the contribution of each ecosystem in explaining variations in contaminant levels within and among species. The Great Lakes are highly disturbed by anthropogenic activities and levels of contaminants are known to be high. The objective of this project was to assess the importance of migration in explaining variation in mercury (Hg) concentrations observed in migratory fish-eating birds. Two migratory piscivorous bird species, the double-crested cormorant (*Phalacrocorax auritus*; short migrant) and the Caspian tern (*Hydroprogne caspia*; long migrant) were caught in Lake Ontario during breeding season and feathers grown during the winter were collected to analyze Hg and stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ) and hydrogen ( $\delta^2\text{H}$ ) isotopes. Preliminary results indicate that a proportion of individuals had higher Hg concentrations than the adverse effect threshold for adult piscivorous birds. Strong positive relationships between Hg and  $\delta^2\text{H}$  suggest that Hg is more bioavailable in southern locations. High Hg values are associated with high  $\delta^{13}\text{C}$  values (marine habitats) and  $\delta^2\text{H}$  values (southern habitats) suggesting high accumulation in those habitats. No clear relationship is observed between Hg and  $\delta^{15}\text{N}$ , suggesting that input of anthropogenic nitrogen and/or trophic level are not significant factors affecting Hg



accumulation. Location where breeding occurred partly explains Hg levels in wintering grounds. This research project helped to understand the effect of migration on Hg levels in aquatic birds and the importance of biovector transport of Hg through birds.

### **Assessment of mercury contamination in African sub-Saharan freshwater reservoirs (Burkina Faso)**

OUSSÉNI, O. <sup>1</sup> and MARC, A. <sup>1</sup>. <sup>1</sup>University of Montreal

Artisanal gold mining with metallic mercury (Hg) amalgamation is a common practice in Africa, and has been on the rise in Burkina Faso since 1990. However, there are no data on the potential impact of these activities on Hg contamination of aquatic systems in Burkina Faso. Total mercury (THg) and methylmercury (MeHg) concentrations were determined in water and 350 muscle tissues of fish samples from 13 reservoirs in Burkina Faso in order to provide a first assessment of environmental mercury contamination. Mercury was analysed by a cold-vapour atomic fluorescence spectrometry technique using Tekran 2600 mercury analyzer (CV-AFS) after oxidization by BrCl and reduction by SnCl<sub>2</sub>. Hg concentration ranged from 0.45 to 17.04 ng L<sup>-1</sup> and 0.02 to 0.071 ng L<sup>-1</sup> for THg and MeHg in water, respectively. Most of the mercury was in particulate form due to rainfall runoff. Fish THg level ranged from 0.002 to 0.607 µg g<sup>-1</sup> (wet weight). Most of the fish mercury concentrations were below the Health Canada guideline limit of 0.2 µg g<sup>-1</sup> (wet weight) for protecting people at risk. Only one fish species (*Bagrus bajad*) had Hg level above the World Health Organization (WHO) international trade guideline limit of 0.5 µg g<sup>-1</sup> (wet weight). These results suggest that, in general, most fish species are not significantly contaminated by Hg, with the exception of *Bagrus bajad*. In order to assess risk and develop policy recommendations, future studies should also consider consumption patterns of different subpopulations.

### **Aqueous concentrations and degradation pathway of methylmercury in Arctic lakes and thaw ponds**

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Photodemethylation of monomethylmercury (MMHg) into inorganic mercury (Hg) in Arctic ponds and lakes represents a major degradation pathway for this neurotoxin. From 2008-2010, fieldwork was conducted on Bylot and Cornwallis Islands (Nunavut) to assess the level of MMHg contamination of aquatic systems and its photodegradation. In 27 ponds and 7 lakes, concentrations in water ranged from 1.0-30.4 ng L<sup>-1</sup> for Hg and 0.02-18.2 ng L<sup>-1</sup> for MMHg. The highest concentrations of MMHg were mostly found in runnels (ponds formed over melting ice wedges) and are considered extreme compared to levels in natural aquatic systems. Incubated natural water exposed to continuous sunlight during arctic summer presented considerably lower concentrations of MMHg than samples incubated in the dark, (losses of 33-38% of MMHg after one week). Higher photodemethylation rates were observed in polygon ponds (formed on peat polygons), whereas lower rates were found in deep-seated runnels, less exposed to sunlight. Additional photodemethylation experiments were performed in the field and the laboratory to examine the role of different solar radiation wavebands, sulfur-containing compounds, and reactive oxygen species on this degradation pathway. We conclude that runnels formed by thawing permafrost constitute hotspots of

MMHg contamination in the Arctic, and that photodemethylation is a significant loss mechanism in northern aquatic systems.

### **Effects of nanosilver on bacterial communities in natural waters**

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Silver nanoparticles (nAg) may be introduced into aquatic ecosystems because of their widespread use as antimicrobial agents. However, the effect of nAg on fundamental processes in the environment is relatively unknown. Bacterioplankton collected from 9 aquatic habitats (3 rivers, 3 stormwater management ponds, and 3 lakes) were exposed for 48 hrs to carboxy-functionalized nAg (ViveNano Inc., 10 nm particle size) at 6 different nominal concentrations. Bacterial responses were quantified as bacterial production and Michaelis-Menten activity of two extractable enzymes, aminopeptidase and alkaline phosphatase. Bacterial production and alkaline phosphatase activity, but not aminopeptidase activity, were significantly reduced in all nAg-exposed samples after only 1 hr of exposure. However, over the subsequent 48-hr exposure period, bacterial production recovered by 40-250% in many of the treatments at lower nominal concentrations (0.05 and 0.1 mg L<sup>-1</sup>) but was still inhibited (~100%) at the highest nominal concentrations (1 and 10 mg L<sup>-1</sup>) compared to the 0 mg L<sup>-1</sup> samples. In positive control tests with AgNO<sub>3</sub>, bacterial production was also completely inhibited at 1 hr of exposure, and this effect persisted throughout the 48 hrs of exposure. These results illustrate that natural microbial communities exposed to nAg in the aquatic environment at environmentally relevant (i.e. µg L<sup>-1</sup>) concentrations may be affected by these exposures, but the effects may be short-lived and compensated for by changes in community composition or by processes that remove nAg from the aqueous phase.

### **Can Microtox be a valuable tool in measuring nanoscale particle toxicity?**

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Although the potential applications of nanoscale particles within consumer and industrial products is seemingly endless, a key question that this industry still has to address is how to measure, using cost effective and high throughput testing protocols, the safety of these particles once they have entered into the environment. Microtox® is an example of such a test that is currently being used extensively by industry to measure toxicity of effluents, products and drilling wastes. To determine whether Microtox® could effectively measure nanoscale particle toxicity, we first optimized Microtox® testing conditions by manipulating testing parameters (pH, age of reagent, methanol addition etc.) to identify the ideal test ranges. These same parameters were then manipulated in the presence of nanoscale particles to identify ideal conditions for testing representative nanoscale particles. In addition to manipulating the testing media, we also evaluated the impact of adding several surface-active agents as dispersants for nanoscale particles on this test system. The results from this study will be discussed.

## Surface chemistry of silica nanoparticles modulates their cytotoxicity to rainbow trout cell lines

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Silica nanoparticles (SiO<sub>2</sub>-NPs) are increasingly becoming used in popular products such as tires, high efficiency photovoltaics, and in many products intended for human usage including toothpaste, sunscreens, cosmetics, nutraceuticals and drug delivery vehicles. It is estimated that about one million tons of SiO<sub>2</sub>-NPs are manufactured worldwide annually. These nanoparticles are eventually discharged into water bodies, potentially threatening aquatic life forms. Surface modification of SiO<sub>2</sub>-NPs has been reported to reduce aggregation and non-specific binding, and detergent-coated SiO<sub>2</sub>-NPs have been shown to be easily removed from sewage compared to uncoated particles. Although uncoated SiO<sub>2</sub>-NPs are generally non-toxic, the toxicity of coated particles has not been fully evaluated. Despite the ease in the removal of coated SiO<sub>2</sub>-NPs, the surface chemistry effects on modulating the toxicity of SiO<sub>2</sub>-NPs in fish is unknown. In an attempt to understand this differential effect, tests were performed with SiO<sub>2</sub>-NPs coated with cetyltrimethylammonium bromide (CTAB) as a surfactant or with different cationic polyelectrolytes such as polybrene and poly(diallyldimethylammonium chloride) (PDDA) on rainbow trout macrophage (RTS-11) and fibroblastic gonadal (RTG-2) cell lines. Cell viability was determined by Alamar Blue, CFDA-AM and Neutral Red assays 24 hours post-exposure. These bioassays respectively measure metabolic activity, membrane integrity, and lysozyme function in cells. Differential responses were observed with the tested cell lines, and responses varied with the type of surface coatings. A dramatic decrease in cell viability was observed when RTS-11 were exposed to SiO<sub>2</sub>-NPs coated with polybrene. RTS-11 appeared to be more sensitive to SiO<sub>2</sub>-NPs coated with cationic polyelectrolytes whereas RTG-2 cells were more sensitive to CTAB coated particles. Surface coatings thus appear to modulate toxicity of SiO<sub>2</sub>-NPs and should be evaluated for impacts on whole fish and possibly other aquatic organisms.

## Change in metallothionein phosphorylation state in *Mya arenaria* clams: Implication in metal metabolism and oxidative stress

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The contamination of the benthic environment poses a threat to long-lived sessile organisms such as clams. The purpose of this study was to investigate metal contamination in tissues and examine changes in metallothioneins (MT) with redox status in *Mya arenaria* clams collected at three polluted sites. The phosphorylation state of MT was also investigated to determine its involvement in cytoprotective signaling by protein kinase C (PKC) during contamination stress. The results show that clams collected from at least one of the polluted sites presented significantly higher concentrations of silver (Ag), arsenic (As), cobalt (Co), copper (Cu), mercury (Hg), nickel (Ni), tin (Sn) and lead (Pb) in their tissues. In the visceral tissue, total MT levels and the reduced, metal-binding form of the protein were significantly induced at the sites. The phosphorylation of MT and mitochondrial activity, as determined by electron transport and cytochrome c oxidase activities, were also significantly reduced at the contaminated sites. Reduced phosphate levels in MT were negatively correlated with total MT levels, suggesting that decreased phosphorylation was involved in phosphate signaling during

cellular stress and could possibly alter the protein's affinity to confer cytoprotection against heavy metal contamination. An evaluation of MT phosphorylation might provide some clues to the hormone-mimicking effects of metals and the modulation of binding affinities during heavy-metal and oxidative stress in clams.

### **The effects of fluoxetine on corticosteroidogenesis in fish**

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Fluoxetine (FLX), the active ingredient of Prozac™, is a member of the selective serotonin reuptake inhibitor (SSRI) class of anti-depressants and is present in aquatic environments worldwide. Previous studies report that FLX is an endocrine disruptor in fish where it concentrates in tissues including the brain. Evidence indicates that activity of the hypothalamo-pituitary-interrenal (HPI) stress axis is influenced by serotonin and thus exposure to FLX may disrupt the teleost stress response. This study examined corticosteroidogenesis (cortisol production) in vitro using an isolated rainbow trout (*Oncorhynchus mykiss*) head kidney preparation. Trout were exposed in-tank to nominal FLX concentrations of 540 ng L<sup>-1</sup> and 54 µg L<sup>-1</sup> for 12 days. Interrenal cortisol production was evaluated after adrenocorticotrophic hormone (1 IU ACTH) induction of the cells in vitro. Results indicate that interrenal cells isolated from trout exposed to 54 µg L<sup>-1</sup> FLX produced significantly less basal and ACTH-stimulated cortisol compared to the control and low FLX-exposed (540 ng L<sup>-1</sup>) trout. Therefore, exposure to FLX may result in reduced corticosteroidogenesis in the trout head kidney, possibly altering the fish's ability to successfully adapt and survive encountered stressors. Further research on all components of the HPI axis will elucidate the precise mode of action by which FLX alters the teleost endocrine stress response. Funded by NSERC, CWN and ON Best in Science.

### **Environmental influences on 17α-ethynyl estradiol uptake and oxygen consumption in fish**

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Pharmaceuticals are not readily removed from the environment through wastewater treatment facilities. The synthetic estrogen 17α-ethynyl estradiol (EE<sub>2</sub>), a common hormone used in birth control and hormone replacement therapy, is very lipophilic, and is thought to have a similar uptake pathway as oxygen across the gills. Both male killifish (*Fundulus heteroclitus*) and juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to radio-labelled EE<sub>2</sub> at a nominal concentration of 100 ng L<sup>-1</sup> for 2 hours at two different salinities (0ppt and 16ppt) to which they had been previously acclimated for 3 weeks. Killifish were also exposed to EE<sub>2</sub> at temperatures of 4, 10, 18 and 26°C. For each EE<sub>2</sub> exposure, the rate of oxygen consumption (MO<sub>2</sub>) was measured while the fish were in individually-sealed respirometers. Killifish displayed a greater uptake of EE<sub>2</sub> in 50% seawater than in freshwater. The distribution of EE<sub>2</sub> was found to be the highest in the carcass, gut and liver with the highest absolute uptake in the 26 and 18°C treatments. MO<sub>2</sub> decreased with decreasing PO<sub>2</sub>, while higher temperatures produced higher MO<sub>2</sub> values. Overall, a positive correlation was found between EE<sub>2</sub> uptake and MO<sub>2</sub> (R<sup>2</sup> = 0.713) in killifish. Investigating temperature, salinity and other environmental parameters will provide insight into the uptake of EE<sub>2</sub> by teleosts under varying environmental conditions. (NSERC Strategic)

### Model androgen effects in mummichog (*Fundulus heteroclitus*)

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Androgens enter the environment from various sources, including veterinary and human pharmaceuticals and pulp mill effluents. Androgens may be aromatizable (i.e., can be converted by animals to estradiol via the aromatase enzyme) or nonaromatizable. To evaluate the response of a native estuarine fish species to androgens, adult mummichog (*Fundulus heteroclitus*) were exposed to static waterborne exposures of 10 µg L<sup>-1</sup> and 100 µg L<sup>-1</sup> nonaromatizable dihydrotestosterone (DHT) and 100 ng L<sup>-1</sup> and 1000 ng L<sup>-1</sup> aromatizable methyltestosterone (MT) in a 14-day reproductive endocrine bioassay. Plasma levels and in vitro gonadal production of testosterone, estradiol and 11-ketotestosterone were measured. In females, significant depressions of plasma estradiol occurred in both DHT treatments as well as the 1000 ng L<sup>-1</sup> MT treatment. In vitro production of estradiol was lower in females exposed to 100 µg L<sup>-1</sup> DHT and 1000 ng L<sup>-1</sup> MT. Gene expression of steroidogenic acute regulatory (StAR) protein and aromatase was evaluated in gonadal tissue. Cytochrome P450 1A (CYP1A), CYP3A, and vitellogenin expression were measured in liver. Results of expression levels for these genes are pending. Preliminary results demonstrate that androgen effects occur primarily in females; expression studies may identify mechanisms responsible for observed effects. These findings can help elucidate risks and mechanisms of action of fish of androgen exposure in the environment.

### Endocrine disruption effects of wood extractives present in Chilean pulp mill effluents on juvenile and embryo fish

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We evaluated endocrine-disruptive effects in juvenile rainbow trout (*Oncorhynchus mykiss*) and embryo toxicity in three species including trout, American flagfish (*Jordanella floridae*) and Japanese Medaka (*Oryzias latipes*) following exposure to wood extractives (Solid Phase Extraction) from Chilean pulp mill effluents (untreated, primary and secondary treated). Triploid and natural juvenile trout were intra-peritoneally injected with all extracts, along with several standards including steroid hormones (E2 and testosterone), phytosterols (β-sitosterol) and resin acid (dehydroabietic acid, DHAA). Fish embryos (8hpf) were waterborne exposed to the same treatments in a semi-renewal system until the first fish hatched, and mortality, hatchability, and malformations were evaluated (fish were maintained until they reached sexual maturation). Results from these experiments indicate that all pulp mill extracts were estrogenic in juvenile trout, resulting in an increase of circulating vitellogenin (VTG) and up-regulated CYP19a gene expression leading to increased endogenous estrogens. Additionally, an anti-estrogenic effect was detected in a subsequent experiment which injected DHAA+E2 simultaneously (resin acids were significantly present in Chilean effluents). No significant mortality with respect to the controls was observed in any of the exposed embryos, in contrast to the significant delay in hatchability observed in embryos exposed to all pulp mill extracts. Increases in developmental abnormalities were detected only in medaka, eventually resulting in embryo mortality. The sex ratio indicated a significant



increase in females, reaching 60-75% of the total survivors for all species of fish. These results indicated that Chilean pulp and paper mill effluents continue to be estrogenic to fish.

#### **Forensic ecotoxicity evaluations: A review**

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<sup>1</sup>HydroQual Laboratories, <sup>2</sup>ALS Laboratory Group

As effluent and water quality requirements are becoming more stringent and new industry sectors are starting to require ecotoxicity testing (e.g. wastewater treatment plants), there is a potential for toxic effects to be found. If toxic effects are found then industry is often required to identify the toxic constituents of effluent or process water and wherever possible, mitigate these effects. Forensic ecotoxicity investigations are designed to isolate, characterize and identify the sample constituents which are toxic to the aquatic test species of interest. A review of several forensic case studies performed in collaboration between HydroQual and ALS Laboratory Group will be discussed to highlight innovative techniques for confirmatory testing and analytical contributions. Several case studies from different source effluents will be explored. The case studies will include general background information about each site, relevant standard and customized toxicity test design and results, and analytical techniques and findings. Some interesting confirmatory techniques explored as part of these investigations include size exclusion testing, pH-adjustment, and ion balance and sulfate toxicity testing. Another issue that makes forensic ecotoxicity investigations challenging is that in many complex effluent mixtures, there is the potential for multiple toxicants to cumulatively contribute to the observed toxic effects. Alternate methods for delineating the relative contributions of these potential toxicants will be discussed.

#### **Behavioural analysis of *Chironomus tentans* and *Lumbriculus variegatus* for use in bio-monitoring of aquatic systems**

SOLNIK, J. <sup>1</sup>. <sup>2</sup>Ryerson University

*Chironomus tentans* and *Lumbriculus variegatus* have been extensively used in the past as bioindicators in aquatic systems. Previous experiments have focused on non-repeatable biological endpoints such as bioaccumulation, growth, mass, and survivorship. The behaviour of these organisms has been repeatedly overlooked despite its ability to provide quick, repeatable, and wide-range identification of toxic compounds in an aquatic system. Due to the inherent difficulty of behaviour quantification there has been little to no research done on the use of behaviour as a monitoring tool. It is the purpose of this study to illustrate the uses of behaviour for utilization in the bio-monitoring of aquatic systems. Both *C. tentans* and *L. variegatus* were exposed to varying concentrations of the herbicide Atrazine and the biocide tributyltin (TBT). These toxins have been prevalent in Southern Ontario water systems due to surrounding agriculture and ship traffic through the Great Lakes and have the potential to enter surrounding aquatic systems, including drinking water sources of the surrounding population. The concentrations of Atrazine used were 5, 50, and 500  $\mu\text{g L}^{-1}$ , while the concentrations of TBT used were 1, 10, and 100  $\mu\text{g L}^{-1}$ . The specific concentrations used for each species were determined by previous studies and environmental relevance. The three behavioural endpoints for *C. tentans* were body positioning, foraging, and burrow construction. The *L. variegatus* endpoints were swimming pattern, body positioning, and clumping. Behavioural responses were captured using time lapsed photography and live video

feed. Under exposure to Atrazine, neither *C. tentans* nor *L. variegatus* elicited an abnormal behavioural response. Under the highest concentration of TBT ( $100 \mu\text{g L}^{-1}$ ) *C. tentans* elicited a reduction in foraging frequency; however *L. variegatus* showed no stress behavioural responses to any concentration of TBT. This study illustrates the usefulness of behavioural responses in the attempt to identify the presence and toxicity of androgenic contaminants in aquatic systems.

### **Multivariate approaches in detecting differences in communities: Is non-metric multidimensional scaling the answer?**

JACKSON, D. <sup>1</sup>. <sup>1</sup>University of Toronto

Community and environmental data generally involve many variables and sites, often necessitating a multivariate approach to determining underlying relationships: e.g., have communities at some sites undergone major changes due to environmental alterations? A commonly used approach is non-metric multidimensional scaling, which provides a method of plotting the relative differences amongst the sampling locations based on the species composition. Many researchers consider this approach to be the superior method to analyze community data and essentially to be free of underlying statistical assumptions. I examine how effectively this method is able to capture underlying patterns that are known a priori. I show that a commonly used approach fails to determine the correct number of axes to incorporate, a fundamental requirement in this method, and is frequently unable to correctly to summarize the relationships. As a consequence, underlying differences in communities may be defined incorrectly and major changes in communities due to environmental impacts may not be detected, or perceived differences may occur when no impacts are known to have happened.

### **Using a toxicological model for hypothesis testing of aquatic toxicity data**

MCCARTY, L. <sup>1</sup>. <sup>1</sup>L.S. McCarty Scientific Research

The validity, utility, and applicability of toxicity test results from standard testing protocols is contingent on whether the employed toxicological model and assumptions are appropriate and sufficient for the circumstances of each test conducted. As an initial attempt to determine the possible consequences of the lack of toxicological model specification and validation, the standard 96 h acute toxicity test protocol was subjected to hypothesis testing. A one-compartment, first-order kinetics model was specified as the toxicological model. The first enabling assumption was that a steady-state LC50 must either be reached or estimated from the observed data. The second enabling assumption is that a constant time to achieving the LC50 is most appropriate. A subset of the U.S. EPA fathead minnow 96 h acute toxicity database was employed. Approximately 8% of the over 350 tests examined failed the first validation step, as steady-state was not reached and could not be estimated. The remainder of the test results examined exhibited times to steady-state that varied by a factor of over 2000 times, from 0.02 to 34 days. These tests failed the second validation step, as the time to achieve LC50 was not constant at about 96h but varied widely. Using exposure-based dose data from unvalidated and uncorrected toxicity test results can introduce substantial variability/uncertainty in estimates of relative toxic potency, even with a single test protocol

with a single species from the same laboratory. Formal adoption of explicit specification of toxicological model and associated assumptions, combined with routine validation, as is currently done for the statistical component of toxicity testing, is clearly required to improve the toxicological utility of toxicity testing data.

### **Does the molecular weight of toxicants determine the survival of activated resting stages in aquatic animals?**

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Three inorganic toxic heavy metals (Cu, Cr, Hg) and two organic (phenol and formalin) chemicals were tested at increasing concentrations as potential toxicants to interfere with embryonic development in the resting stages of *Ephydatia* sp. (Demospongia), *Plumatella fungiosa* (Bryozoa), *Daphnia galeata* (Daphniiformes), *Wlassicsia pannonica* (Macrothricidae), *Artemia salina* (Anostraca), *Moina macrocopa* (Moinidae), *Lymnadia lenticularis* (Conchostraca) and *Heterocypris incongruens* (Ostracoda). To test the hypothesis whether smaller sized molecules can more easily penetrate protective membranes, we used ionized oxygen (H<sub>2</sub>O<sub>2</sub> and ozoniser CERTIZON C25, producing 25 mg O<sub>3</sub> h<sup>-1</sup>) and ammonium ion (NH<sub>3</sub>) (atomic weight 16 a.u. and 15 a.u. respectively).

Lower resistance of embryos of different taxa inside protective membranes coincided with smaller molecular weight in organic toxicants, or with smaller ion atomic weight in toxic heavy metals. The maximal damage (mortality 98%) was observed in experiments with ozone and ammonium.

High resistance of the resting stages to toxic heavy metals and organic toxicants within a long time of treatments (up to 90 days) possibly let some species survive during periods of heavy pollution of sea ports, oil terminals etc. This study was supported by RFBR grant 07-04-00006 and bilateral Russia-Taiwan grant 05-04-90588.

### **Acute and chronic toxicity of two formulations of the pyrethroid pesticide deltamethrin to amphipods, sand shrimp and lobster larvae (PO)**

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Pyrethroid insecticides are among the most toxic insecticides known, and marine crustaceans are generally more sensitive to pyrethroids than marine fish. Our objectives were to measure toxicity to marine crustaceans of two deltamethrin formulations in sea water using conventional 96-hr test methods, 1-hr pulse exposures and chronic 14- to 16-day exposures. The agricultural formulation (Decis) is applied to various crops and has potential to enter aquatic habitats via spray drift or runoff. The aquaculture formulation (AlphaMax) is applied directly to skirted salmon sea cages to kill sea lice, and is then subsequently released to the estuarine or coastal marine environment. The marine organisms tested were: *Homarus americanus*, the American lobster; *Crangon septemspinosa*, the sand shrimp; and *Eohaustorius estuarius*, a benthic amphipod. Results showed stage III lobster larvae had acute 96-hr LC<sub>50</sub> for formulated deltamethrin between 3.74 to 4.92 ng L<sup>-1</sup>. Stage IV lobster had 96-hr LC<sub>50</sub> of 28.2 ng L<sup>-1</sup>. Stage III lobster larvae given a 1-hr pulse exposure followed by 16 days in

clean water, had an  $LC_{50}$  of  $36.5 \text{ ng L}^{-1}$ . Chronic exposure of stage III lobster larvae for 16 days had an  $LC_{50}$  of  $4.45 \text{ ng L}^{-1}$ . The amphipod *E. estuarius* had acute 96-hr  $LC_{50}$  for formulated deltamethrin between  $1.66$  to  $7.99 \text{ ng L}^{-1}$ .  $EC_{50}$  for the same tests were from  $<0.32$  to  $<3.2 \text{ ng L}^{-1}$ . *E. estuarius* given a 1-hr pulse exposure followed by 95 hrs in clean water had an  $LC_{50}$  of  $13.3 \text{ ng L}^{-1}$ , and an  $EC_{50}$  of  $5.52 \text{ ng L}^{-1}$ . When *E. estuarius* was given a 48-hr pulse followed by 48 hrs in clean water the  $LC_{50}$  was approximately  $0.32 \text{ ng L}^{-1}$  with an  $EC_{50}$  of  $<0.032 \text{ ng L}^{-1}$ . Sand shrimp had acute 96-hr  $LC_{50}$  for formulated deltamethrin from  $27.4 \text{ ng L}^{-1}$  to  $45.3 \text{ ng L}^{-1}$ . Sand shrimp given a 1-hr pulse exposure followed by 95 hrs in clean water had an  $LC_{50}$  of  $142 \text{ ng L}^{-1}$ . Chronic exposure of sand shrimp for 14 days had  $LC_{50}$  from  $15.1$  to  $23.8 \text{ ng L}^{-1}$ , with  $EC_{50}$  from  $10.4$  to  $>32 \text{ ng L}^{-1}$ .

#### **Mixture toxicity of herbicides on *Lemna gibba* and recovery potential after prolonged exposure (PO)**

MOHAMMAD, M. <sup>1</sup>, ITOH, K. <sup>1</sup> and SUYAMA, K. <sup>1</sup>. <sup>1</sup>Shimane University, Japan

Combination of several herbicides is usual in the aquatic environments. We examined toxicity of three combinations of herbicides to *Lemna gibba*: paraquat + atrazine, paraquat + alachlor, and paraquat + cyclosulfamuron. The mixture effects were evaluated by comparing the actual relative growth rate (RGR) with the expected RGR calculated from the individual RGR, on a basis of the effects of long-term exposure and recovery potential from the damage. Paraquat and atrazine mixture: The actual RGR was smaller than the expected RGR in both exposure and recovery. The phytostatic concentration was the mixture of 2.5 ppb of paraquat and 100 ppb of atrazine for a 28-day exposure, while it was  $>10$  ppb and 800 ppb for paraquat and atrazine, respectively, for the same period of exposure. Paraquat and alachlor mixture: The expected and actual RGR were almost the same in exposure, while in recovery, the actual RGR was smaller than the expected RGR. Therefore, the joint effects showed stronger synergistic effects in recovery than in exposure. Paraquat and cyclosulfamuron mixture: The RGR in recovery decreased with higher concentrations or prolonged exposure. The mixture toxicity caused lethal effects when the exposure was prolonged beyond 14 days at 10 ppb of paraquat and 0.15 ppb of cyclosulfamuron, which had individually no phytocidal effect. The joint effects often appeared stronger than the expected ones, and could not be predicted from the individual toxicity. In addition, the effects of long-term exposure to herbicides and recovery potential of duckweed were also affected by their combination.

#### **Fertilisation and hatching success of Atlantic cod (*Gadus morhua*) eggs when exposed to various concentrations of produced water (PO)**

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The fertilisation and hatching success of Atlantic cod (*Gadus morhua*) eggs when exposed to various concentrations of produced water obtained from a natural gas production platform was assessed. Eggs were fertilised in sea water (control group, 0%) and four concentrations of produced water: 0.32%, 1.08%, 3.60% and 12% (v/v). The eggs were then held in the respective concentrations for 24 hours and the fertilisation success assessed. Viable fertilised eggs were transferred to 96 well plates containing fresh sea water and incubated in the dark at  $ca 6^{\circ}\text{C}$  until hatch. Eggs started hatching around 14 days post fertilisation (DPF), peaked at around 17 DPF, then finished hatching by 21 DPF. The diluted produced water used for fertilising and holding the eggs for 24 hours will be analysed for total

PAH content using synchronous scan spectrofluorimetry. Results indicated that at 0.32%, 1.08% and 3.6% (v/v), the fertilisation success was greater than or equal to that of the control (0%) group. For the 12% dilution, there were no viable fertilised eggs present after the 24h period. Survivability of eggs during sea water incubation was similar for the 0%, 0.32% and 1.08% treatment groups (1.7% to 3.5% mean cumulative mortality). However for the 3.60% group, egg mortality increased to ca 61.5% mean cumulative mortality at 6 DPF then remained constant. The mean hatching success for all concentrations examined was determined to be 94.5%, 93.1%, 93.1% and 34.0% for the control group (0%), 0.32%, 1.08% and 3.6% (v/v) produced water dilutions respectively.

### **Effects of 4-nonylphenol, cypermethrin, deltamethrin, glyphosate, imidacloprid and mancozeb on growth of Atlantic salmon (*Salmo salar* L.) (PO)**

LYONS, M. <sup>1</sup>, MACKEIGAN, K. <sup>1</sup>, WONG, D. <sup>1</sup>, FAIRCHILD, W. <sup>1</sup> and BURRIDGE, L. <sup>1</sup>. <sup>1</sup>Fisheries and Oceans Canada

Individually PIT-tagged Atlantic salmon (*Salmo salar* L.) smolts were exposed, in fresh water, to pulse-doses of 4-nonylphenol (NP), a putative endocrine disrupting chemical, or one of five current-use pesticides: cypermethrin, deltamethrin, glyphosate, imidacloprid or mancozeb, then transferred to seawater. The smolts were measured (length and weight) at three times: July, September and November. Poor growth has been linked to reduced survival and fewer returns of adult salmon to their native streams. Specific growth rate for weight (SGRW) was affected by some pesticide treatments: in July, fish exposed to imidacloprid (90 mg L<sup>-1</sup> and 10 µg L<sup>-1</sup>) and glyphosate (650 µg L<sup>-1</sup> and 65 µg L<sup>-1</sup>) had significantly lower SGRW compared to controls. Fish exposed to deltamethrin (0.59 µg L<sup>-1</sup>) and glyphosate (650 µg L<sup>-1</sup>) and measured in September showed a significant reduction in SGRW. By November there were no significant differences between treated and control fish for all treatments. Comparisons between the frequency distributions of SGRW in control and treated smolts showed that in July, 4-NP (20 µg L<sup>-1</sup>), imidacloprid (90 mg L<sup>-1</sup>, 8.6 mg L<sup>-1</sup> and 10 µg L<sup>-1</sup>), glyphosate (3000 µg L<sup>-1</sup>, 650 µg L<sup>-1</sup> and 65 µg L<sup>-1</sup>) and mancozeb (50 µg L<sup>-1</sup>) treated fish were significantly different from controls. By November only fish exposed to imidacloprid (90 mg L<sup>-1</sup>) had a significantly different frequency distribution from controls.

### **Studying the anti-estrogenic and liver metabolic effects of DHAA in rainbow trout (PO)**

PANDELIDES, Z. <sup>1</sup>, ORREGO, R. <sup>1</sup>, GUCHARDI, J. <sup>1</sup> and HOLDWAY, D. <sup>1</sup>. <sup>1</sup>University of Ontario Institute of Technology

Pulp and mill effluents contain wood extractives, such as phytosterols, that may cause reproductive disturbances in fish. However, recent studies have shown that dehydroabietic acid (DHAA), a resin acid present in pulp and paper mills, may have anti-estrogenic effects in fish. A chronic-exposure toxicity experiment using immature rainbow trout (*Oncorhynchus mykiss*) was conducted in order to assess the endocrine disrupting and liver metabolic effects of wood extractives regularly present in pulp and paper mills, such as DHAA,  $\beta$ -sitosterol (BS) and model estrogen 17 $\beta$ -estradiol (E2). The effect of these compounds was evaluated by analysis of plasma vitellogenin (VTG) levels, gonadal somatic index (GSI), liver somatic index (LSI), liver ethoxyresorufin-O-deethylase (EROD) activity, liver lactate dehydrogenase activity (LDH), liver citrate synthase activity (CS) and serum sorbitol dehydrogenase activity (SSDH),



respectively. The experimental procedure involved the use of multiple intra-peritoneal (IP) injections (1 injection every 7 days for an exposure period of 28 days). It is hypothesized that the phytosterols BS and E2 will show an induction of EROD and VTG levels after 7, 14 and 28 days exposure. Furthermore, it is expected that simultaneous injection of DHAA + E2 and DHAA + BS will reduce the VTG levels found in fish injected with E2 or BS alone. Results will be discussed.

#### **Bioavailability and toxicity of priority azo dyes to benthic invertebrates and turtles (PO)**

MILANI, D.<sup>1</sup>, DE SOLLA, S.<sup>1</sup>, BALAKRISHNAN, V.<sup>1</sup>, MARTIN, P.<sup>1</sup>, WEBBER, J.<sup>1</sup>, THOMPSON, S.<sup>1</sup> and LEGAULT, D.<sup>1</sup> <sup>1</sup>Environment Canada

The environmental occurrence and impact of dyes are emerging issues that have attracted significant attention. Azo dyes (Chemicals Management Plan (CMP) medium priority listed dyes) are synthetic dyes that are considered to be inherently toxic, bioaccumulative, and/or persistent. Some dyes may bioaccumulate in wildlife due to their moderate or high octanol-water partition coefficient, particularly in organisms that live or feed from benthic environments. Supplemental research includes an ongoing project funded under CMP, where methods are being developed to determine concentrations and degradation rates of azo dyes in water, sediment, and biotic tissues. Data gaps that remain to be addressed include the need for additional physical properties for use in "read-across" models, estimates of sorption-desorption coefficients in sediments and soils, tissue extraction method development and the determination of bioavailability and toxicity of dyes to benthic and terrestrial organisms. This study addresses the toxicity and bioaccumulation data gaps by 1) determining the toxicity of dye-contaminated sediment to the benthic invertebrates *Hexagenia* spp. (mayfly) and *Tubifex tubifex* (oligochaete worm); 2) measuring the effect of soil contaminated with dye to development (hatching success, deformities, growth) of turtle embryos; and 3) determining bioaccumulation-sediment/soil accumulation factors. To date, benthic invertebrates and turtle eggs have been exposed to Sudan Red G in a series of concentrations. Methodologies and some preliminary results (i.e., lethal concentrations resulting in 20% and 50% mortality, point estimates for sublethal endpoints) will be presented. Future work and other potential azo dyes to be tested will be discussed.

#### **Impact of size and aggregation state on the cytotoxicity of cadmium telluride quantum dots (PO)**

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<sup>1</sup>Environnement Canada, <sup>2</sup>NRC Biotechnology Research Institute, <sup>3</sup>University of Montreal

Quantum dots (QDs) are synthesized nanoparticles (NPs) of great commercial value for microelectronics and biomedical applications. The increasing demand for and commercialization of these and other nanomaterials is raising concerns about their potential release to aquatic environments. The purpose of this study was to examine the cytotoxicity of functionalized QDs to rainbow trout hepatocytes (RTH) and HepG2 cells and, given that CdTe QDs can form aggregates of different sizes, to determine if the size distribution of nanoparticles aggregates may modulate their cytotoxicity and the leaching of cadmium. Rainbow trout (*Oncorhynchus mykiss*) hepatocytes and HepG2 cells were exposed to increasing concentrations of different size distributions of CdTe Quantum dots or dissolved

cadmium (CdCl<sub>2</sub>) for 48 h at 15°C. Results indicate that CdTe QDots were cytotoxic to both RTH and HepG2 cells in a dose-dependent manner at concentrations in the mg L<sup>-1</sup> range. A significant difference in toxicity depending on the size and distribution of aggregated particles was observed. RTH were able to discriminate 3 classes of toxicity while HEPG2 cells discriminated 2 classes of toxicity. In both cell systems, fractions of smaller-sized particles exhibited somewhat higher potency.

### **Investigating the effects of the sea lice pesticide emamectin benzoate on clam worms (*Nereis virens*) (PO)**

MCBRIARTY, G.<sup>1</sup>, KIDD, K.<sup>1</sup> and BURRIDGE, L.<sup>2</sup>. <sup>1</sup>University of New Brunswick Saint John, <sup>2</sup>Fisheries and Oceans Canada

*Nereis virens* (Clam worms) may be cultured beneath ocean-based Atlantic salmon aquaculture pens serving as an organic extractive species in Integrated Multitrophic Aquaculture (IMTA). These worms have commercial value as bait but may be exposed to antilousing compounds, such as emamectin benzoate (EB, trade name SLICE®), that are put into salmon feed and can persist in organic sediments beneath cages for over a year. As no current information exists on the effects of EB on *N. virens*, preliminary tests were conducted to develop methods and to get a rough estimate of its acute toxicity to the worms. Subsequently, a definitive acute toxicity experiment was conducted using a series of 10-day trials, with worms being exposed individually to a nominal concentration range of 0, 2.58, 7.8, 24, 72, 218, 660, 2000 µg kg<sup>-1</sup> (wet weight) of EB in sediment; actual sediment concentrations will be determined via HPLC analysis. These exposures will be used to determine a lethal concentration (LC50) and body burdens of EB, as well as effects on the size and behaviour of the worms. Threshold toxicity concentrations identified from these studies will be compared to concentrations of EB that have been reported to occur in the environment around aquaculture sites to assess its potential risk to *N. virens* in an IMTA system.

### **Use of immunohistochemistry with frozen sections for vitellogenin detection in year-2 class natural population rainbow trout (*Oncorhynchus mykiss*) (PO)**

KRAUSE, R.<sup>1</sup>, HOLDWAY, D.<sup>1</sup> and GUCHARDI, J.<sup>1</sup>. <sup>1</sup>University of Ontario Institute of Technology

Vitellogenin (Vtg) is used as a biomarker of exposure to estrogenic xenobiotics found in the environment. Produced in the liver, this protein is detected in the blood using an enzyme-linked immunosorbent assay (ELISA). Once in the gonadal tissue, it can be detected within cells using immunohistochemistry (IHC) with paraffin wax or frozen sections. The traditional paraffin wax method has been criticized due to extensive tissue processing which can compromise antigen integrity; frozen IHC can preserve antigens for antibody recognition. This project analyzed gonadal tissue of twelve sexually mature rainbow trout using paraffin and frozen histology. The two techniques were compared for efficacy and section quality. The method for detecting Vtg with IHC on frozen sections was optimized. The IHC results were qualitatively compared to blood Vtg levels, as well as the known sex and maturation stage of each fish. Frozen sectioning was a useful method with less tissue processing, and yielded sections which were comparable to paraffin wax sections. IHC was effective at detecting Vtg within oocytes with fluorescence intensity correlating to oocyte maturity. Frozen sectioning

IHC will be useful for detecting Vtg induction in gonadal tissue of rainbow trout exposed to estrogenic xenobiotics in both environmental and laboratory settings.

### **Methodological opportunities to quantitatively interpret environmental media: A myriad of choices (PO)**

*CHOROSTKOWSKA, E. <sup>1</sup>, FITZGERALD, D. <sup>1</sup> and HARPER, B. <sup>1</sup>. <sup>1</sup>Trow Associates Inc.*

A large range of methods exists to allow for the quantitative interpretation of environmental media, such as water concentrations relative to aquatic screening guidelines. Such methodological options include: 1) simple numeric comparisons of media to established government guidelines; 2) development of simple statistical description of media for comparison against government guidelines; 3) use of detailed statistical analyses such as linear regression or non-linear curve fitting of environmental media; 4) use of environmental media to estimate site-specific guidelines for use in government metrics such as the water quality index (WQI); and 5) derivation of alternative hazard concentration 5% (HC5) to estimate site-specific guidelines with the species sensitivity distribution (SSD) approach. Various factors come into play when selecting the methodology best suited to interpret environmental media, such as seasonal fluctuations, adjacent land use, and other temporal and spatial variations. This presentation will demonstrate these methodological opportunities through the use of long-term environmental observations of chloride for three types of watersheds—urban, agricultural and forested—located across Canada. The demonstration of these methods provides an opportunity for more informed choices of the methods available for such analyses.

### **Distribution and abundance of benthic macroinvertebrates in lakes in Kejimikujik National Park, Nova Scotia (PO)**

*NUSSBAUMER, C. <sup>1</sup>, BILYJ, B. <sup>2</sup> and BURGESS, N. <sup>1</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>BIOTAX*

Environment Canada's Acid Rain Biomonitoring Program in Atlantic Canada was implemented in 2009 with the purpose of establishing the current composition and abundance of aquatic invertebrate species in acid-sensitive lakes within and around Kejimikujik National Park, Nova Scotia. A total of 20 lakes which range in pH from 4.3 to 6.0 were surveyed during the month of June in 2009 and 2010. All sampled lakes were oligotrophic, and many were shallow with rocky or boulder shorelines. Each lake was sampled for littoral benthic and nektonic macroinvertebrates, and specimens collected from 9 lakes in June 2009 have been identified down to species level where possible. Specimens collected from a further 11 lakes surveyed in June 2010 have yet to be identified. From the results obtained thus far, a total of 165 macroinvertebrate taxa have been identified. Macroinvertebrate diversity was reduced with increased levels of acidity, and some macroinvertebrate groups such as Mollusca and Hirudinea do not occur in acidic lakes (pH < 5). These results provide a first look at macroinvertebrate biodiversity in acid-sensitive lakes within Kejimikujik National Park area, and will feed into an ongoing assessment by Environment Canada of impacts of acid deposition on aquatic wildlife in eastern Canada.

## **Determining aquatic toxicity thresholds under the Environmental Emergency Regulations (PO)**

*BOURGEOIS, J. <sup>1</sup> and SHRIVES, J. <sup>1</sup> <sup>1</sup>Environment Canada*

The Environmental Emergencies Division at Environment Canada is responsible for regulating chemicals at fixed facilities to prevent and prepare for potential spills, based on their level of risk and potential for exposure to human life or health. Under the Environmental Emergency Regulations (E2 regs), the potential for inhalation toxicity and the likelihood of explosion were the predominant risk criteria for substances to be recommended for addition to the E2 regs. Following the publication of the E2 regs in 2003, awareness was raised about the detrimental effect that acutely toxic chemicals could have on the aquatic environment or its biological diversity. Therefore, the Environmental Emergencies Division has developed a novel methodology to determine aquatic toxicity thresholds for hazardous substances, based on acute aquatic toxicity, bioaccumulation and persistence criteria, in order to assess the risk of potential spills into the aquatic environment. This poster will summarize the development of this novel risk assessment methodology and its implementation under the E2 regs (Part 8 of CEPA, 1999).

## **If all of your friends used $\alpha = 0.05$ . . . would you do it too? (PO)**

*MUDGE, J. <sup>1</sup>, HOULAHAN, J. <sup>1</sup>, EDGE, C. <sup>1</sup> and BAKER, L. <sup>1</sup> <sup>1</sup>University of New Brunswick, Saint John*

One of the most intuitive and well known problems associated with hypothesis testing using designated alpha levels is the arbitrariness of the alpha-value selected. Despite this, there have been infrequent and sporadic attempts to develop defensible approaches to setting alpha levels (the probability of type 1 error—i.e., making a false positive conclusion) in environmental science. The practice of using one or a few standard alpha levels with little or no regard for the relative probabilities and/or costs of false positive (type 1) and false negative (type 2) errors has no logical foundation. This is particularly true for fields related to environmental science, upon which natural resource management decisions are often based, and which can have species-specific costs of species decline and conservation. An appropriate alpha level should depend on the context of the study, and be set to minimize the overall probability of making a wrong conclusion, with possible adjustments made for the relative costs of type 1 and 2 errors and differences in choice of critical effect size. A simple, flexible tool will be presented that ecotoxicologists will be able to use to set appropriate alphas that minimize the overall probability of making a wrong conclusion, while incorporating explicit decisions about the relative costs of type 1 and 2 errors and critical effect sizes. Proposed use of this tool will be demonstrated using several ecotoxicology datasets.

## **The effects of glyphosate-herbicides on zooplankton communities and the emergence of adult insects from wetlands (PO)**

BAKER, L. <sup>1</sup>, KIDD, K. <sup>1</sup>, HOULAHAN, J. <sup>1</sup> and THOMPSON, D. <sup>2</sup>. <sup>1</sup>University of New Brunswick Saint John, <sup>2</sup>Natural Resources Canada

Glyphosate-based herbicides are some of the most commonly used herbicides in the world. These are generally considered to have a low direct toxicological risk to animals, but few studies have attempted to investigate the ecosystem-level effects of formulated glyphosate products in conjunction with other likely co-occurring environmental contaminants. Small wetlands are ubiquitous in agricultural and forestry landscapes, and have considerable potential for contamination. Thirty-four wetlands, each split with an impermeable barrier to allow for direct comparison between treated and untreated halves, were used to examine the effects of glyphosate-based herbicides on wetland invertebrates. Barriers were installed in wetlands located on Canadian Forces Base Gagetown, near Oromocto, NB; treatments were applied in spring 2009 in 24 agricultural-scenario ponds and in the fall of 2009 in 10 forestry-scenario ponds. Five preservative-free emergence traps were deployed on each side of each wetland, before and after treatment, to collect emerging insects. Five zooplankton samples were collected before and every five days after for up to 90 days post treatment, to measure the effects on the abundance and community composition of the zooplankton community. Emergent insects are dependent on the structure of the plant community. With changes in the plant community caused by the herbicide treatments, it is predicted that there will be a significant change in the emergence of adult insects from the affected half of each pond. Zooplankton have been shown to be sensitive to glyphosate-based herbicides and are predicted to show decreases in abundance due to the herbicide treatments.

## **The use of eel (*Anguilla rostrata*) cell lines in an ecotoxicological problem: The dramatic decline in eel populations (PO)**

BLOCH, S. <sup>1</sup>, BROMAND, S. <sup>1</sup>, WALSH, S. <sup>2</sup>, WONG, J. <sup>1</sup>, LEE, L. <sup>2</sup>, HODSON, P. <sup>3</sup> and BOLS, N. <sup>1</sup>. <sup>1</sup>University of Waterloo, <sup>2</sup>Wilfrid Laurier University, <sup>3</sup>Queen's University

The American eel (*Anguilla rostrata*) is experiencing a significant population decline. One hypothesis is that increased selenium levels in the Great Lakes might play a role in this population decrease. In this study we propose to use cell lines derived from eel to explore selenium toxicity. Selenium is naturally found in metal ores, including copper, silver, and lead. Industry and other human activities are sources of selenium in aquatic ecosystems. It quickly attains threatening levels to wildlife and was recently recognized as being responsible for different fish kills, such as in Belews Lake (North Carolina) and Kesterson Reservoir (California). The use of fish cell lines can be useful to elucidate toxicity mechanisms, to rank chemical compounds for toxicity, and to assess toxicant loads in eels. Cells derived from the brain of adult eels and gill from juvenile eels were used in short-term cytotoxicity tests with sodium selenite, sodium selenate and selenomethionine. Sodium selenite and sodium selenate are the most common form of inorganic selenium found in the environment. Selenomethionine is the most common organic form found in the environment, and it is considered to be the most threatening form to wildlife because of its high bioavailability and its high potential to bioaccumulate. In the short-term cytotoxicity test the toxicity ranking of



the three compounds was sodium selenite > sodium selenate > selenomethionine. This ranking corresponds also to acute toxicity tests on whole fish. Selenomethionine was used in cell growth experiments, where it showed to inhibit cell growth over a longer time period.

#### **TIE for cyanides in groundwater at a former coal gasification plant (PO)**

MCLEAY, M. <sup>1</sup>, CAMERON, M. <sup>1</sup> and ELPHICK, J. <sup>2</sup>. <sup>1</sup>Hemmera, <sup>2</sup>Nautilus Environmental

Groundwater beneath a former coal gasification plant site being remediated in British Columbia contains high levels of cyanides, as well as other substances with concentrations exceeding aquatic life guidelines. Hemmera and Nautilus Environmental undertook a study to determine whether that groundwater was toxic to a variety of sensitive marine aquatic life species, and whether cyanides were the primary toxicants. Information gathered will be used in planning how to treat/discharge excavation water during site remediation, and/or as part of an ecological risk assessment for the site.

Untreated groundwater containing 500 µg L<sup>-1</sup> total cyanide, 29 to 38 µg L<sup>-1</sup> weak acid dissociable cyanide (WAD), and <5 µg L<sup>-1</sup> free cyanide, was tested for toxicity using the following standardized tests: 48-h bivalve (*M. galloprovincialis*) larval survival/development; 48-h kelp (*M. pyrifera*) zoospores germination/growth; sea urchin (*S. purpuratus*) gamete fertilization success; and 7-day larval topsmelt (*A. affinis*) survival and growth. Untreated groundwater was found to be toxic to kelp zoospores and sea urchin gametes, but relatively non-toxic to bivalve larvae and topsmelt. Four toxicity identification evaluation (TIE) treatments were conducted on site groundwater. These included: 1) acidification/aeration of the sample; 2) filtration of the sample through anion exchange media; 3) filtration of the sample through activated carbon; and 4) exposure of the sample to UV light. The anion exchange treatment significantly reduced toxicity to kelp and reduced cyanide concentrations in the sample. These findings provided support for a conclusion that the toxicity may be wholly or partly attributed to cyanides in the groundwater.

#### **A tiny problem: Developing the techniques for quantifying the toxicity and uptake of carbon nanotubes to soil organisms (PO)**

MARTIN, W. <sup>1</sup>, ANGELL, R. <sup>2</sup>, SHRIVE, E. <sup>2</sup>, STEPHENSON, G. <sup>2</sup>, TANG, S. <sup>1</sup> and BOLS, N. <sup>1</sup>.

<sup>1</sup>University of Waterloo, <sup>2</sup>Stantec Consulting Ltd.

Nanoparticles such as carbon nanotubes promise to revolutionize medical and engineering science and research, with applications in industry that include medical treatment, sporting equipment, and improved computing technologies. The properties that make them so promising are also responsible for the concerns that have arisen regarding the safety of these materials to both human health and environment. These same properties also make traditional toxicological testing methods less effective, and the quantification of nanotube uptake and persistence problematic. Experiments were conducted in an attempt to overcome some of the challenges of assessing nanotubes in soils. The toxicity of carbon nanotubes to earthworms was determined without using a highly toxic dispersant used in past experiments. A stock "slurry" composed of sand, water, ethanol (which evaporates before exposure), and multi-walled carbon nanotubes was formulated in order to incorporate the nanotubes and achieve a homogeneous distribution within soils. The quantification of nanotubes within both the soil and test organisms was accomplished through the use of thermogravimetric analysis and raman spectroscopy.

### **Effects of total suspended solids on macroinvertebrate benthic communities in Arctic inland lakes (PO)**

VANENGEN, R. <sup>1</sup>, SIBLEY, P. <sup>1</sup>, MANN, G. <sup>2</sup> and SOLOMON, K. <sup>1</sup>. <sup>1</sup>University of Guelph, <sup>2</sup>Azimuth Consulting Group

As the mining industry continues to grow in the Canadian Arctic, there is a need to evaluate the sensitive aquatic environments that dominate the landscape, are culturally significant and are valued fisheries resources. We evaluated the effects of increased inputs of Total Suspended Solids (TSS) on benthic invertebrate communities and sediment quality in Second Portage Lake (65°00'51"N: 96°05'33"W) following 2008 mine-related, in-water dike construction where concentrations of TSS exceeded the CCME guideline of 25 mg L<sup>-1</sup> in parts of the lake. During the summer of 2009, we completed a field study to evaluate the macrobenthic community, beginning with comparisons of taxa richness and total abundance in exposed stations in Second Portage Lake vs. reference stations. One-way ANOVA followed by a Tukey's pair-wise comparison found there were statistically significant ( $p < 0.05$ ) differences among exposed areas (stations that were inside impermeable turbidity curtains used to contain TSS) compared to internal and external reference stations. Effects on benthic endpoints generally followed a gradient with fewer changes away from the source. Follow-up benthic invertebrate studies and sediment toxicity are being conducted and preliminary results will be presented.

### **Caractérisation minéralogique de sédiments de surface de la rivière Richelieu, Québec, Canada (PO)**

JAOUICH, A. <sup>1</sup>, KARAM, A. <sup>2</sup> and LAFOND, J. <sup>1</sup>. <sup>1</sup>Université du Québec à Montréal, <sup>2</sup>Université Laval

La caractérisation minéralogique permet de connaître la nature minéralogique des phases constituant les sédiments et de mieux évaluer le comportement des contaminants dans les sédiments des rivières et des fleuves, et les démarches de gestion environnementale possible. En 2005, une campagne d'échantillonnage de sédiments s'est déroulée pour caractériser les sédiments de surface à l'embouchure de la rivière Richelieu à Sorel-Tracy (Québec, Canada). L'analyse chimique des sédiments révèle la présence de plusieurs éléments traces (Cr, Cu, Pb, Zn, Ni et Hg) et de polluants organiques (HAP et hydrocarbures pétroliers). La phase géochimique la plus active pour la majorité des échantillons est la phase carbonatée. Les minéraux identifiés par diffraction aux rayons X sont : carbonate de calcium, carbonate de magnésium, quartz, kaolinite, illite, chlorites, amphiboles, smectites, feldspath potassique, plagioclases, apatite, hématite et pyrite. L'analyse minéralogique semi-quantitative de deux fractions granulométriques (<2µm et <10µm) montre la prédominance de l'illite et des chlorites par rapport à la kaolinite et aux smectites. Le contenu en phyllosilicates de la fraction <2µm (33,5%) est supérieur à celui de la fraction <10µm (28,7%). Les chlorites sont ferrières et présentes dans la matrice de sédiment sous forme trioctaédrique. Les sédiments ayant une texture argilo-silteuse semblent montrer une altération géochimique plus prononcée que ceux de texture sablonneuse. Les résultats de l'analyse minéralogique et chimique permettent de conclure que les sédiments faiblement contaminés peuvent être traités et valorisés en milieu terrestre.

### **Switchable surfactants: A toxicity assessment of an emerging class of surfactants (PO)**

ARTHUR, T.<sup>1</sup>, PHAN, L.<sup>1</sup>, HARJANI, J.<sup>1</sup>, JESSOP, P.<sup>1</sup> and HODSON, P.<sup>1</sup> <sup>1</sup>Queen's University

For both environmental protection and improved energy efficiency, switchable surfactants have been developed to change polarity and solubility upon command. Surfactant activity can be turned on by introduction of one atmosphere of CO<sub>2</sub> and reversed by purging with air. These surfactants have a variety of potential industrial applications related to their ability to separate oil from other mediums and their ability to destabilize emulsions. We conducted a series of experiments to test the acute toxicity of switchable surfactants to 1-3g Rainbow Trout (*Oncorhynchus mykiss*). The compounds were tested at pH ~8.0 to evaluate their toxicity in natural surface waters. The surfactants were produced in several variations, differing in composition by hydrophobic tail group, hydrophilic head group, or both. A strong correlation between the estimated octanol/water partition coefficient ( $K_{ow}$ ) and the toxicity of 8 compounds generated a structure-activity relationship that was used to design a compound that combines lower toxicity with surfactant properties. This model shows that despite the addition of varying functional groups, switchable surfactant toxicity remains largely dependent upon  $K_{ow}$  and differs little from traditional surfactants of equivalent structure.

### **The role of urban wetland community structure and function in contaminant fate (PO)**

GILBERT, N.<sup>1</sup> and KIRKWOOD, A.<sup>1</sup> <sup>1</sup>University of Ontario Institute of Technology

Wetlands have a unique capacity to attenuate pollution through various biological, chemical and physical means. However, not much is known about the functional capacity of urban wetlands to process contaminants. The objectives of our study are to characterize the community structure and function of different urban wetland communities and compare their biodegradation efficiencies. Three types of urban wetlands in Oshawa, ON, were investigated, including a remnant wetland, constructed wetland and contaminated remnant wetland. Each wetland type is considered unique and predicted to have a different capacity to attenuate pollution due to differences in physicochemical properties, biological community structure and previous contaminant exposure. Wetlands were characterized based on the following physicochemical properties: pH, salinity, organic matter and soil particle size distribution. The catabolic potential of the microbial communities present at each site was assessed using the BiologEco™ plates method. Differences in average well color development (AWCD) indicated a difference in catabolic potential between microbial communities of each site. Results from these analyses show unique community profiles for each wetland. Microcosm experiments are currently being conducted to investigate the ability of each wetland community to attenuate a common organic pollutant (the herbicide 2,4-dichlorophenoxyacetic acid) using UPLC and UV detection to measure rates of removal. Results from these ongoing experiments will be presented at the conference.

### **Application of SPME LC-MS/MS for analysis of drugs in fish bile for biomonitoring purposes (PO)**

*TOGUNDE, P. <sup>1</sup>. <sup>1</sup>University of Waterloo*

Solid phase microextraction (SPME), a simple sample preparation method, was developed and validated for screening target pharmaceuticals in immature rainbow trout (*Oncorhynchus mykiss*) bile samples following 14 d of aqueous exposure to carbamazepine and fluoxetine in the laboratory. SPME offers the distinct advantage of integrating sampling and sample preparation into a single step, thereby reducing multiple analytical steps during sample preparation. This method is suitable for small sample volumes (< 100 µL), and consequently can be used on fish bile samples without dilution. Both fluoxetine and carbamazepine were bioconcentrated in the bile, indicating both compounds were taken up by fish from water with the bioconcentration factor of fluoxetine significantly higher than that of carbamazepine. As the less polar compound, greater Phase II conjugation was evident for fluoxetine with detected concentrations increasing substantially after de-conjugation with sulfatase, glucosidase, and glucuronidase. This study demonstrates application of SPME as a useful analytical technique capable of monitoring the target compounds and their respective target metabolites in fish biological fluid such as bile.

### **The analysis of naphthenic acids by LC/MS/MS using Q-TOF mass spectrometry as a detection method (PO)**

*HINDLE, R. <sup>1</sup> and NOESTHEDEN, M. <sup>1</sup>. <sup>1</sup>Vogon Laboratory Services Ltd.*

Naphthenic acids are a complex class of carboxylic acids found as contaminants in tailing pond waters in Alberta's oil sands. These natural chemicals are concentrated during the hot-water extraction of bitumen from the oil sands. The analysis of naphthenic acids can be carried out by a variety of techniques. Gas chromatography with mass spec detection requires derivatization, while mass spec coupled to HPLC does not. A popular technique with LC/MS is the use of flow injection analysis (FIA), where the sample is injected directly into the source without any separation of the acids or matrix compounds. This work will show a comparison of FIA analysis to that carried out with gradient chromatography, and demonstrate whether ion suppression effects are reduced. Analysis of the naphthenic acid mixture with mass resolution between 10-20,000 was used to determine the molecular formulae, using electrospray ionization and quadrupole time-of-flight (Q-TOF) detection with 2-ppm mass accuracy.



### The effects of mercury on yellow perch (*Perca flavescens*) reproductive and general health in Kejimikujik National Park and Historic Site, Nova Scotia (PO)

FISCHER, K. <sup>1</sup>, KIDD, K. <sup>1</sup>, MUNKITTRICK, K. <sup>1</sup>, DREVNICK, P. <sup>2</sup> and BURGESS, N. <sup>3</sup>. <sup>1</sup>University of New Brunswick - Canadian Rivers Institute, <sup>2</sup>Université du Québec - INRS-ETE, <sup>3</sup>Environment Canada

Mercury (Hg) contamination of aquatic ecosystems and the accumulation of methylmercury (MeHg) in wildlife and humans, and subsequent health effects, are global concerns. Kejimikujik National Park and National Historic Site (KNPNHS) is a relatively remote and pristine area situated in southwestern Nova Scotia, with no point sources of Hg. However, high Hg concentrations are found in common loons (*Gavia immer*) and their main prey, yellow perch (*Perca flavescens*), and for this reason KNPNS has been designated a Hg "hotspot". Concentrations of total Hg in yellow perch in KNPNS have increased over the last decade, and many currently exceed the tissue threshold effect level of 0.2 µg Hg g<sup>-1</sup> (ww) for juvenile and adult fish. To determine whether elevated Hg levels are negatively affecting the general and reproductive health of this species in KNPNS, mature male and female yellow perch were collected in the fall of 2009 from 6 lakes known to contain fish with low, medium or high Hg concentrations. The following health variables are being examined in these fish: condition; liver-somatic index (LSI); gonadosomatic index (GSI); histology (gonads, liver, spleen and kidney); concentrations of plasma sex steroids (testosterone, 11 keto-testosterone, and 17β-estradiol); expression of stress-related genes in the liver. Mean total mercury concentrations in skinless dorsal muscle of these perch ranges from 0.27 - 0.63 µg Hg g<sup>-1</sup> (ww) (n = 21 to 43/lake) for all sizes. A high occurrence of macrophage aggregates in liver, kidney and spleen tissues was found in perch with high muscle Hg concentrations, indicating that tissue damage is occurring in these fish. However, significant among-lake differences in condition and GSI health measures do not appear to be related to Hg concentrations in the fish. Based on these results, Hg-related effects do not appear to be occurring at the organism level, but observed tissue damage in the liver, kidney and spleen may be related to Hg. Additional endpoints are still being examined, and 3 additional lakes will be sampled in September 2010.

### Induction of phytochelatins in *Chlorella vulgaris* by exposure to selenate and the effect of the sulphate ion (PO)

SIMMONS, D. <sup>1</sup> and EMERY, R. <sup>2</sup>. <sup>1</sup>Waterloo University, <sup>2</sup>Trent University

Phytochelatins (PCs) are short peptides made from the precursor molecule, glutathione (GSH). There has been little evidence to confirm the ability of the anion selenium (Se) to induce phytochelatin production. Therefore a goal of this study was to examine whether Se could induce phytochelatin production in algae. Furthermore, it is thought that selenate competes with sulphate in the sulphur assimilation pathway and sulphate demonstrates a protective effect against the toxic effects of high doses of Se in algae. Hence, the interaction of selenate and sulphate was investigated with respect to the induction of PCs. *Chlorella vulgaris* was cultured in media with either low - 3 mg L<sup>-1</sup> or high 30 mg L<sup>-1</sup> sulphate. These cultures were exposed to selenate in doses of 1, 5 and 10 µg L<sup>-1</sup> for 48 hrs. In a separate treatment cadmium was added as a positive control to induce PCs, and there was one no-



metal exposure (negative control). Quantification of selenate and other Se species, GSH, PC 2 and PC 3 were measured in the cell medium. PCs and GSH were induced by Se and the positive Cd control. There was a marked effect by sulphate whereby PCs and GSH were significantly greater for all metal treatments in the low sulphate groups, and PC production was significantly inhibited in the high sulphate groups. These data suggest the existence of a negative feedback system in the sulphur assimilation pathway affecting phytochelatin production and also support the competition for uptake at the ion transport level between selenate and sulphate.

### **The effect of cations on toxicity of Ni to duckweed (*Lemna minor*) (PO)**

GOPALAPILLAI, Y. <sup>1</sup>, VIGNEAULT, B. <sup>2</sup> and HALE, B. <sup>1</sup>. <sup>1</sup>University of Guelph, <sup>2</sup>Natural Resources Canada

Major cations (Ca, Mg, Na, K) are generally known to reduce toxicity of Ni by competing for the biotic ligand in aquatic organisms such as fish. Plants are designed to take up lots of macronutrients such as Ca and Mg. Most soil plant studies have shown that increasing Ca and Mg does not show a competitive reduction in Ni uptake. This may be because micronutrients and macronutrients have different sites of entry into the plant, which is likely controlled by homeostasis. This homeostatic control of nutrient uptake by plants further confounds a study of cation effect on trace metal toxicity in plants. For example, previous studies have suggested that when an element is essential (such as Ni), the detoxification system may not kick in immediately as it would during exposure to a non-essential element such as Cd. In the present study, the effect of adding various cations on Ni toxicity was examined in the aquatic plant *Lemna minor*. Explanations for the observed effects were supported by Ni accumulation data. The results show that the major cations do not competitively reduce Ni toxicity at environmentally relevant concentrations. Kinetic analysis shows an anti-competitive effect of Ca on Ni toxicity. Hence, we suggest that toxicity of Ni in aquatic plants is dominated by homeostatic control and a model based on simple competitive cation effects on trace metal toxicity in aquatic plants is not sufficient. This is similar to findings in terrestrial plants.

## **Urban Stormwater**

### **Spatial vs. temporal variations of the toxicity in stormwater ponds: Evidence obtained by combining bioassessment methodologies**

TIXIER, G. <sup>1</sup>, FROST, P. <sup>2</sup>, ROCHFORD, Q. <sup>1</sup>, GRAPENTINE, L. <sup>1</sup>, MARSIALEK, J. <sup>1</sup> and LAFONT, M. <sup>3</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>Trent University, <sup>3</sup>Cemagref

Stormwater ponds have been widely used to control increased surface runoff resulting from urbanization and to enhance runoff quality. As receiving waters, they are impacted by intermittent stormwater pollution and also serve as newly created aquatic habitats, which balance the transformation of aquatic ecosystems and their biodiversity in urban areas. Thus, determining ecological risks in stormwater ponds is crucial for the preservation and rehabilitation of biodiversity in urban areas. Limitations of the conventional techniques for assessing the complex toxicity patterns in stormwater ponds have led us to adopt an

integrative approach combining two bioassessment methodologies: the sediment quality triad and an oligochaete index-based method developed by the Cemagref (Lyon, France). The combined methodology has been applied at eight sites in the Terraview-Willowfield stormwater facility in Toronto, Ontario, in all four seasons (summer 2008 – spring 2009). Sediment chemistry results indicate that several contaminants (among PAHs and heavy metals) exceeded the Probable Effect Limit (Canadian Sediment Quality Guidelines) in the facility. Regardless of the season, laboratory bioassays revealed strong spatial variation in sediment toxicity along the flow path from the inlet to the outlet, agreeing with decreasing concentrations of contaminants in sediment, especially of heavy metals. However, *in situ* assessments of the benthic macroinvertebrate community structure and the oligochaete indices revealed an overriding influence of seasonally varying toxicity, which was highest in the spring and lowest in the fall.

### **Development of a Canadian Water Quality Guideline for chloride for the protection of aquatic life**

*NOWIERSKI, M. <sup>1</sup> and FLETCHER, T. <sup>1</sup>. <sup>1</sup>Ontario Ministry of the Environment*

The application and storage of road salts (of which NaCl is the most widely used) for snow and ice control in the winter is a major anthropogenic source of chloride to the aquatic environment. Approximately 2,950,000 tonnes of chloride were released into the Canadian environment as a result of road salt application in the winter of 1997/1998; over one-third of this was released in Ontario. The Ontario Ministry of the Environment has been monitoring chloride concentrations in Ontario tributaries since 1964. Data from 1990 to 2005 showed levels of chloride in rivers and streams ranged from non-detect to a maximum measurement of 5,080 mg L<sup>-1</sup>. Elevated concentrations of chloride associated with deicing have been documented in groundwater, wetlands, streams, and ponds adjacent to snow dumps and salt-storage areas, and also those receiving significant run-off from roadways and urban areas. Elevated chloride concentrations have the potential to adversely affect freshwater ecosystems, and so there is a strong need to develop water quality guidelines for chloride for the protection of aquatic life. A synopsis of literature-based toxicity data will be presented, as well as an overview of draft short- and long-term guidelines developed for chloride using species sensitivity distributions. A discussion on how these values compare to chloride measurements in the field will be presented.

### **Re-thinking stormwater management pond sediment disposal options: Landfill waste or soil amendment resource?**

*KELLY-HOOPER, F. <sup>1</sup>. <sup>1</sup>University of Waterloo*

The Ontario Water Resources Act includes stormwater management facilities (SWMFs) under the "Sewage Works" definition. In the case of Sewage Treatment Plants (STPs), treated sewage sludge may be used as a beneficial agricultural soil amendment material if it meets the "Guidelines for the Utilization of Biosolids and Other Wastes on Agricultural Land". At the present time, however, SWMF sediments are managed as waste materials under the Ontario Environmental Protection Act. Sediment chemistry concentrations exceeding the O. Reg. 153/04 Brownfield soil standards would be disposed of at registered landfill facilities. This waste disposal approach is currently practiced throughout Canada and most of the United States. Thousands of cubic meters of excavated/dredged sediment can cost hundreds of

thousands of dollars in landfill tipping fees, while also consuming valuable landfill space. During the period of 2005-2009, a sediment chemistry field survey was conducted on 34 SWMFs located in five Canadian provinces. The results of this chemistry study indicate that 33 of the 34 SWMF sediments met the most stringent biosolids and composting guidelines. A pilot study is currently underway in Ontario that proposes to utilize site-specific SWMF sediments as non-agricultural topsoil and/or compost amendment materials. The primary objective of this pilot study is to demonstrate the steps that would be necessary to obtain MOE approval for the beneficial use of SWMF sediments collected from actual sites. The results of this ongoing pilot study are relevant to current and future SWMF sediment maintenance projects throughout Canada.

#### **Assessing ecological impacts of land-applied municipal biosolids: Effects of run-off and tile drainage on *Daphnia magna* and *Hyalella azteca***

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The effect of run-off and tile drainage from land-applied municipal biosolids on aquatic organisms *Daphnia magna* and *Hyalella azteca* was analyzed through a series of standardized eco-toxicological tests. Treatments included elutriate (run-off and leachate) from reference soil, and reference soil amended with biosolids from two municipal wastewater treatment plants. Elutriate was collected after a simulated storm event 1 day and 40 days after application. Elutriate from soil amended with biosolids was diluted with elutriate from reference soil to create a series of 0, 1, 10, and 100% biosolids elutriate. Respiration rates, behavioural analysis and survivorship were tested on the two invertebrates. In respiration bioassays there was no effect of elutriate from treatment plant 1 at either time point or at any concentration. Elutriate from plant 2 resulted in lower respiration rates in both invertebrates on Day 1 in 10% and 100% concentrations, but there was no effect on respiration at any concentration on Day 40. High concentrations of biosolids elutriates also caused some changes in invertebrate behaviour on Day 1, but there were no effects on behaviour of either invertebrate by Day 40. The experiment found that any adverse effects of elutriate from municipal biosolids were temporary (i.e. only observed in elutriate collected 1 day after biosolids application) and only seen at biosolids elutriate concentrations with little (10%) or no (100%) environmental relevance. The results suggest current regulation and management practices for biosolids land application in Ontario are probably sufficient for protection of invertebrates in water receiving run-off and leachate.

## Freshwater Aquaculture

### Nutrient flow in a freshwater food web during and after an experimental aquaculture operation

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Environmental concerns, including excessive nutrient input from wastes, have limited the growth of freshwater aquaculture in Canada and motivated research on its potential environmental effects. Aquaculture fishmeal has marine ingredients, giving it a distinct enriched  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  when compared to freshwater  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , which allows us to trace the incorporation of dissolved or particulate aquaculture waste into biota. At the Experimental Lakes Area, a cage aquaculture ran for five years before being removed to compare pre- aquaculture and recovery conditions. We examined the movement of fish-farm nitrogen and carbon throughout the lake during its recovery phase to determine whether native organisms are still assimilating aquaculture wastes two years after cage removal. Stable isotope analyses results were compared to the previous four years of data for this lake and a reference system. Both pelagic and littoral invertebrates'  $\delta^{15}\text{N}$  have continued to increase since the end of aquaculture operations, with increases of 2‰ and 1‰, respectively. Minnow  $\delta^{15}\text{N}$  went from 6‰ before to 9‰ during aquaculture and remained at 11‰ for the two years after cage operations ceased. Profundal Chironomidae  $\delta^{15}\text{N}$  has remained at levels similar to those of the last two years of fish production. The  $\delta^{15}\text{N}$  signatures' lack of decrease towards pre-aquaculture levels shows that the nutrients from the cage culture are still being used by the biota in the experimental lake, likely because of recycling of these elements within the food web.

### The role of water hardness in toxicity of nitrate: Implications for water quality guidelines

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The BC Ministry of Environment recently revised its water quality guideline for nitrate to 3 mg  $\text{NO}_3\text{-N L}^{-1}$  for long-term exposures, consistent with the CCME guideline for this anion. Recent studies have shown that the toxicity of sulphate and chloride is reduced with increasing water hardness and, consequently, the current study was performed to determine whether nitrate toxicity is also affected similarly by hardness. Tests conducted included chronic toxicity tests using early life stages of rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*), as well as an amphipod, *Hyaella azteca*, and cladoceran, *Ceriodaphnia dubia*. The early life-stage test using rainbow trout demonstrated a high degree of sensitivity to nitrate, consistent with previously reported data for lake trout. Decreased toxicity was observed with increasing water hardness for each of the species, indicating that it is likely appropriate to consider water hardness as a modifying factor in establishing water quality guidelines.

### **Toxicity evaluation of plant-derived fish-feed components using salmonid intestinal cell lines**

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More than 70% of aquacultured products in Canada are represented by Atlantic salmon and Rainbow trout. Production of these species relies heavily on fish meal diets but the rising costs of this product have prompted the use of cheaper alternative protein sources such as soymeal. Although similar in amino acid composition, the presence of antinutritional components in plant-derived products has limited their utility in salmonid feed formulations. Testing the suitability of various plant fractions in fish is costly, time consuming and unreliable as responses may vary with the physiological status, age or strains of fish. An additional problem for establishing an alternative diet is evaluating the bioactivity of stabilizers and microbiocidals which may be added to feed as well as potential adventitious toxins such as pesticide residues that may get carried on into the feed product. In vitro techniques offer a novel approach to initial testing of feed components for nutritional suitability, as high throughput capacity, reduced costs, fewer animals killed and potential in vivo predictive values can be achieved. The application of two recently established salmonid cell lines, RTgutGC from rainbow trout (*Oncorhynchus mykiss*) intestinal lining cells and ASimf from Atlantic salmon (*Salmo salar*) intestinal myoepithelial cells, for evaluating the bioactivity of various feed components will be presented. The effects of soyasaponins, soybean lectins, tannic acid and phytoestrogens, genistein and daidzein on cell viability and monolayer permeability will be presented.

### ***Ecotoxicology & Endocrine Disruption in Amphibians & Reptiles***

#### **Control and chemical disruption of amphibian development and reproduction**

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Amphibians are particularly sensitive to environmental pollutants and endocrine-disrupting chemicals (EDCs), yet they are often overlooked in ecotoxicological assessments. In some estimates, less than 5 percent of the ecotoxicological literature is dedicated to amphibians. This is surprising given their ecological significance and recent dramatic global population declines. Little is known about the effects of EDCs in amphibians and reptiles, other than in turtles. As an example of University-Environment Canada collaborations we will present an overview of the "why and how" of amphibian Ecotoxicology and Wildlife Health Program at EC. We will also present some recent data on the effects of pesticides on amphibian sexual development. Moreover, the increasing use of native anuran species in ecotoxicological research has exposed the need to develop captive breeding methods and lab culture methodologies, and we will also present some new work in this area.



## Herbicides in the environment and implications for amphibians

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Atrazine and glyphosate are the most widely used herbicides on a global basis. Although interest in the effects of herbicides on amphibians is relatively recent, the studies of amphibians to date have led to consideration of the potential broader ecosystem impacts of herbicides that have not been fully examined in the past. Many studies have examined their toxicity to a variety of species, yet what we still really want to know is, throughout a given year or lifetime of a species, how much herbicide are they exposed to in the wild within ponds? What are the effects on amphibians and/or the aquatic food web they depend on in the wild? The possible link between alterations in immunity and increased parasitic infections in amphibians due to herbicide could have profound and global implications for amphibian survival. And despite the number of researchers and papers recently published on these topics, the effects of atrazine on sexual development and the toxicity of glyphosate to amphibians and their communities remain risks worthy of further research.

## Potential indirect effects of glyphosate herbicides on wood frogs via altered breeding habitat quality

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Glyphosate-based herbicides may impact amphibians through direct lethal or sublethal mechanisms, via indirect effects on ecological structure and function, or both. Previous studies demonstrate that direct toxic effects are unlikely under realistic environmental exposure regimes. More subtle indirect effects on system ecology are plausible and arguably equally important to sustainable local populations. A three-year study conducted in boreal forest regeneration sites of northern Ontario examined potential indirect effects of typical operational aerial herbicide treatments (Vision® or Forza® at nominal rates approximating 2.0 kg a.e. ha<sup>-1</sup>). The experimental design involved a total of 22 monitoring areas (10 treated and 12 untreated) subscribing a number of small ephemeral pools being used intensively by breeding populations of wood frogs (*Lithobates sylvatica*). The study incorporated both temporal and spatial replication and involved assessment of breeding habitat quality using a number of comparative metrics including number, persistence and utilization of breeding pools, water temperature, dissolved oxygen, amount and type of vegetation and occurrence of other anuran species as potential predators or competitors as well as other factors. Preliminary analysis of data derived from two years of intensive post-treatment bioacoustic monitoring, exhaustive egg mass survey, and in-situ egg-hatch success as well as early growth and development of larva showed no significant differences between treated and untreated monitoring areas in either of the two regeneration site types.

**Is exposure to agricultural run-off from row crop agriculture leading to sexual disruption in northern leopard frogs (*Rana pipiens*)? A case study from southwestern Ontario**

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In 2003–2005, surveys of wild male Northern leopard frogs (*Rana pipiens*) in southern Ontario in areas of intensive row crop agriculture were found to have a high proportion of testicular ovarian follicles (TOFs: an average of 45% in extreme Southwestern Ontario) compared to non-agricultural sites which had a much lower incidence (7%). There was no difference in testes size or stage of spermatogenesis between males from agricultural versus non-agricultural sites or between intersex and normal males. We tested male frogs for the presence of vitellogenin (Vtg), an egg yolk precursor normally found in females, as a further indication of endocrine disruption: only a single male frog was found to contain it, and of 23 males with TOF, none expressed Vtg. To determine if the presence of TOFs was linked to exposure to water-borne chemicals from agricultural activity, we took eggs from a non-agricultural site where no intersex individuals had been detected, and raised them in four intensive agricultural sites to determine if the gonadal abnormalities compared to those raised in two non-agricultural sites. The water at the enclosure locations was sampled regularly for analysis of contaminants and pesticides. Eggs and tadpoles were raised in outdoor enclosures until metamorphic climax, when they were removed to the laboratory and euthanized for histological. Overall the proportion of males with ovo testes was significantly higher in agricultural sites versus reference sites ( $p=0.012$ ). There was a significant difference in gonad index (gonad/body wt) in both male ( $p < 0.001$ ) and female ( $p=0.001$ ) frogs among sites. Gonads were significantly smaller in non-agricultural sites than in at least one agricultural site. The presence of TOFs was included in assessments of reproduction and deformities in Great Lakes Areas of Concern (AOCs: St. Clair River and Detroit River) having largely agricultural watersheds. The occurrence of TOFs in AOC sites was significantly greater than in upstream reference sites in Lake Huron and was similar to those seen in non-AOC agricultural waterbodies.

**Biomolecular sensors for determination of deleterious effects in North American frog species**

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Amphibia, particularly frogs, are regarded as important environmental sentinel species yet they are under-utilized for risk assessments. Appropriate tools for assessing sublethal effects of exposure to emerging chemicals of concern such as personal care products, pharmaceuticals, and nanomaterials are needed, particularly for relevant anuran species residing in North American ecosystems. We have been actively engaged in developing biomolecular tools and innovative approaches amenable for use in laboratory and field settings. These include the use of tail fin biopsies in situ and in culture, gene expression arrays, quantitative real-time polymerase chain reaction, protein and metabolite analyses. These approaches will be highlighted along with examples of their utility through practical applications.

### Characterization of the biological and phenotypic effects of 17 alpha-ethynylestradiol exposure during early development in *Xenopus laevis*

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Estrogenic chemicals in the environment have been implicated in causing a variety of adverse effects in exposed organisms. Exposure to estrogens, especially at critical times during sexual development, can cause feminization and/or demasculination of males of many species. 17 $\alpha$ -ethynylestradiol (EE2), a hormone analog commonly used in oral contraceptives, is an estrogenic chemical of environmental concern. EE2 has well-documented and drastic effects on *Xenopus laevis* sexual differentiation and development. At low doses, EE2 can sex reverse genetically male tadpoles to phenotypic females. Using EE2 as a model compound and DM-W, a recently discovered *X. laevis* sex-linked gene, as an indicator of genetic sex, an exposure was performed to attempt to discover the mechanisms underlying the biologically relevant phenomenon of sex-reversal. *X. laevis* were exposed to 0.1, 1, and 10  $\mu\text{g L}^{-1}$  EE2 from 12 h post-oviposition through 13 wks post-hatch. These concentrations span the full spectrum of concentrations previously reported to cause male-to-female sex reversal. All concentrations of EE2 tested in the current experiment significantly delayed completion of metamorphosis. Exposure to 1  $\mu\text{g L}^{-1}$  EE2 caused a significant decrease in weight at termination of the experiment. In addition, phenotypically determined sex-ratios were significantly female-biased in all EE2 treatments but not in controls. Genotyping of EE2-exposed phenotypic female froglets indicated that genetic sex-ratios of these animals was about 60% female and 40% male, which did not differ from sex-ratios in control treatments. DM-W genotyping also allowed definitive classification of EE2-exposed phenotypic female froglets as genetic males for the first time in an experiment of this type. Illumina-based transcriptome analysis of samples to determine the molecular mechanisms underlying sex reversal is ongoing.

### The influence of pond water on the synergistic toxicity of low-dose nanomaterials (MNs) and Roundup WeatherMax<sup>®</sup> to *Lithobates* (Rana) tadpole species

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The regulation of contaminants is often dependent upon controlled within-laboratory toxicological studies that often do not reflect conditions found in the natural environment. Aquatic factors such as varying concentrations of natural organic matter and pre-existing contaminants can greatly alter the toxicity of commonly applied pesticides through synergistic chemistry. In a previous study we observed additive synergistic toxicity between nanomaterials (MNs) and Roundup WeatherMax<sup>®</sup> (RW); however, the influence of dissolved organic carbon (DOC) on the behaviour of RW- and carboxy-coated MNs had yet to be tested. In this study we looked at the acute and chronic toxicity of Roundup to wild North American amphibian species exposed under controlled laboratory procedures and compared it to LC50 values generated by varying natural pond water DOC levels alone and in the presence of low doses (0.1 ppm) of titanium and silver carboxy-coated MNs (ViveNano products; diameter 2-10 nm). Under laboratory conditions Roundup WeatherMax<sup>®</sup> toxicity varied with tadpole species (1.25-3.14 mg FAE L<sup>-1</sup>), with *Lithobates sylvaticus* being the most sensitive and *L. pipiens* being least sensitive. *L. sylvaticus* tadpoles exposed to RW at 0.7 mg FAE L<sup>-1</sup>

throughout development showed similar size and metamorphic rates as the control group, but under histological observation, thyroid and gonadal abnormalities were observed. Looking at the influence of NOM and MNs on *L. pipiens* we found the acute toxicity of RW to increase with increased concentration of DOC (EC50 values 3.79 - 3.46 mg FAE L<sup>-1</sup> for 5 and 15 mgC L<sup>-1</sup>, respectively). No additional acute toxicity was observed with low doses of MNs, but a continued trend with increasing DOC was observed in these treatments. We will continue to look at the influence of various pond waters on MN and RW acute toxicity while examining the influence of DOC and MN on the chronic toxicity of Roundup WeatherMax® to North American amphibian species. This research adds to the discussion of the influence of DOC on the toxicity of commonly used pesticides, highlights sensitive species and aquatic ecosystems, and helps to explain sources of variation between studies used for pesticide regulation.

### Toxicity of deicing salt components to early amphibian life stages

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Chemical contamination is a primary factor contributing to worldwide amphibian declines. Road salt is a major pollutant in northern latitudes, producing elevated chloride concentrations in freshwater systems. Additionally, ferrocyanide anti-caking agents are released from road salt and effects on the environment are poorly understood. Amphibians exhibit low tolerance to salt. Our purpose is to investigate chronic, sub-lethal NaCl exposure on developmental amphibian stages and determine acute toxicity of cyanide. Chronic toxicity experiments at environmentally significant salt concentrations were conducted on larvae of three species and embryos of five species in the laboratory. Species tested were spotted salamanders (*Ambystoma maculatum*), American toads (*Anaxyrus americanus*), spring peepers (*Pseudacris crucifer*), green frogs (*Lithobates clamitans*) and wood frogs (*Lithobates sylvaticus*). Cyanide acute toxicity experiments were performed on the five species. Chronic salt exposure reduced hatching, increased mortality, and induced developmental and behavioural anomalies. Median lethal concentration values were calculated from cyanide acute toxicity experiments. Results indicate that the chronic effects can inflict detrimental consequences on amphibian populations.

### The Long-term Experimental Wetlands Area (LEWA): Providing evidence from natural systems

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The Long-term Experimental Wetlands Area (LEWA) was established at CFB Gagetown in New Brunswick in 2008. The objective of LEWA is to provide an opportunity for replicated, whole-system experiments on small and mid-sized wetlands. LEWA is approximately 8 square kilometers of young mixed forest and early successional scrubland with 100–200 ponds and wetlands of varying sizes. We have currently established 2 large-scale sets of experiments and an acoustic amphibian monitoring program and have a third set of experiments addressing climate change impacts proposed to begin in spring 2011. The first experiment uses a split-pond design and examines the effects of 1) RoundUp WeatherMax® and New VisionMax®, two glyphosate-based herbicides designed for agricultural and forestry applications,



respectively, and 2) nutrients/interspecific competition on plant, phytoplankton, invertebrate and amphibian communities. We measure endpoints from gene expression to species richness. The second experiment also uses a split-pond design and examines the impact of amphibian density (primarily wood frog density) on ecosystem dynamics. The proposed climate change set of experiments will use a paired-pond design and examine the effects of temperature increases between 2 and 6 degrees C on plant, phytoplankton, invertebrate and amphibian communities. There will be a series of talks at this session presenting preliminary results of the multiple stressor experiment. LEWA would not have been possible without the support and co-operation of the Department of National Defence and collaborators from UNB Saint John, the Canadian Rivers Institute, the University of Ottawa, the Canadian Forest Service, and Environment Canada.

### **Modelling the effects of fertilizers and herbicides on wetland food web structure**

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Amphibians are important consumers in aquatic and terrestrial ecosystems. Their populations are in decline worldwide, and agricultural contaminants in wetlands may be part of the cause. Glyphosate-based herbicides and fertilizers are common contaminants in wetlands, but are not known to be directly toxic to larval amphibians at environmentally relevant concentrations. However, these chemicals can have dramatic effects on the wetland ecosystem by causing significant impacts on emergent plant species and aquatic algae. The aquatic larval stages of amphibians are primary consumers of algae and detritus. This can lead indirect impacts on amphibian species, mediated by changes in the structure and function of the entire food web. Six wetlands, each split with an impermeable plastic barrier to allow for direct comparison between treated and untreated halves, were used to examine the effects of glyphosate-based herbicides and inorganic fertilizers on the structure of the wetland food web. Barriers were installed in wetlands located on Canadian Forces Base Gagetown, near Oromocto, NB, in summer of 2008; treatments were applied during spring and summer of 2009. Adult and larval amphibians, aquatic invertebrates, plants, algae and detritus were collected from both sides of each wetland at the end of the summer of 2009. Using stable isotope analysis and gut contents, a model of the food web was created, which could be compared to its counterpart in the untreated half of each wetland to understand whether these contaminants affect the structure of food webs in wetlands.

### **The interactive effects of a glyphosate herbicide (RoundUp WeatherMax™) and nutrient enrichment on amphibian survival, development, and growth in naturalized wetlands**

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Glyphosate-based herbicides are the most widely used herbicides in the world. Recent laboratory and mesocosm studies have shown these herbicides can be lethal to amphibian larvae at sufficiently high concentrations. However, field-based studies have demonstrated that when applied at environmentally realistic concentrations, glyphosate-based herbicides have little to no effect on amphibian survival, growth or development. Amphibian larvae develop in a variety of wetlands where they are may be concurrently exposed to multiple



biotic and abiotic factors which could exacerbate or mitigate herbicide effects. For example, in agricultural regions wetlands experience nutrient enrichment as a result of fertilizer runoff, which can cause eutrophication potentially acting as a stressor or alternatively enhancing microbial degradation of the herbicide. We investigated the effect of a widely used glyphosate-based herbicide (RoundUp WeatherMax™) and nutrient enrichment on wood frog (*Lithobates sylvaticus*) survival, growth and development in naturalized ponds split in half using an impermeable plastic barrier. We did not observe any effect when the herbicide was applied at the environmentally observed concentration (EOC: 0.21 mg a.e. L<sup>-1</sup>). At the predicted maximum environmental concentration (PMEC: 2.89 mg a.e. L<sup>-1</sup>) we observed no effect on survival, and limited evidence for accelerated development. However, when combined with nutrient enrichment, animals metamorphosed at larger body sizes at both herbicide application rates, and developed at a faster rate in the PMEC treatment.

### **Phenology of amphibian breeding in relation to pesticide exposure in Ontario**

PALONEN, K. <sup>1</sup>, DE SOLLA, S. <sup>1</sup> and STRUGER, J. <sup>1</sup>. <sup>1</sup>Environment Canada

Amphibians often are exposed to pesticides while breeding in agricultural landscapes, and the timing of breeding may affect their exposure. The phenology of amphibian lifestage (egg, tadpole, metamorph, adult) varies among frog species, as do their behaviours (calling, breeding, post-breeding). Our objective was to identify the relative exposure to pesticides for each lifestage and behaviour. Using frog-calling intensities, time to hatch, and time to metamorphosis for eight frog species across Ontario, we estimated the lifestage and behavioural phenologies. Data from Environment Canada's Pesticide Science Fund initiative in Ontario were used to quantify temporal changes in concentrations of pesticides in surface waters. For American toads, leopard frogs and spring peepers, the pesticide concentrations generally increased throughout their three life stages, whereas for bullfrogs and green frogs, exposure was highest during the egg stage. Generally the concentrations peaked post breeding for early breeders and during breeding for late breeders, and thus pesticide residues were highest during the tadpole or metamorph stage for late breeders. Data relating pesticide exposure with amphibian lifestage and breeding behaviour is important for assessing risk, and for designing appropriate exposure regimes for toxicological studies.

### **In situ kinetics of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) in green frog tadpoles (*Rana clamitans*) (PO)**

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A study was conducted to determine *in situ* variation of chemical exposure dynamics in tadpoles. Green frog tadpoles (*Rana clamitans*) were dosed with Performance Reference Compound polychlorinated biphenyls (PRC PCBs) in order to simultaneously measure *in situ* chemical uptake and elimination rates. Performance reference compound PCBs are analytically non-interfering organic compounds that are selected to match the physicochemical properties of chemicals of interest. Tadpoles were caged and placed into agricultural, municipal and reference water bodies near Windsor, Ontario, Canada. Performance reference compound PCBs were eliminated significantly faster than in previous laboratory studies, with elimination rate coefficients ranging between 0.200 and 1.295 day<sup>-1</sup>. Elimination rate constants of PRC PCBs were not significantly different ( $P > 0.05$ ) among sites;

therefore metabolic activities of tadpoles were similar and they were able to achieve steady state with their different environments within as little as 22 days. This similarity in chemical toxicokinetics of tadpoles in different environments indicates that tadpoles are excellent biomonitors, reflect environmental contamination and provide an accurate indication of *in situ* chemical inputs and concentrations.

### Effects of glyphosate-based herbicides on genes involved in the control of metamorphosis of wood frogs (*Rana sylvatica*) (PO)

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Glyphosate-based herbicides are widely used across the world and they have been shown to affect survival and developmental rates of tadpoles. Increased use of these herbicides in the past decades may be a contributing factor to the global decline of amphibian populations. The purpose of this study is to determine if glyphosate-based herbicides affect the expression of specific genes involved in metamorphosis of wood frogs tadpoles (*Rana sylvatica*). We hypothesized that exposure to glyphosate-based herbicides will affect developmental rates by disrupting hormone pathways. Tadpoles were chronically exposed to different concentrations of VisionMax®, a glyphosate-based herbicide, in a laboratory setting and sampled at different stages of development. Results show a 31.3% increase in mortality and a decrease in developmental rates of tadpoles exposed to 2900 µg L<sup>-1</sup> of VisionMax®. Gene expression analysis indicated that this high concentration significantly increased the expression of thyroid hormone receptor beta by 20 folds ( $p=0.001$ ) and deiodinase type 2 by 16 folds ( $p=0.02$ ) in tails, as well as deiodinase type 3 by 29 folds in brains ( $p=0.033$ ) at metamorphic climax. We demonstrate that exposure to 2900 µg L<sup>-1</sup> of VisionMax® alters metamorphosis in developing tadpoles by disrupting the thyroid axis.

### Relationship between pesticide properties and absorption in turtles eggs from treated soil (PO)

DE SOLLA, S. <sup>1</sup> and MARTIN, P. <sup>1</sup>. <sup>1</sup>Environment Canada

Our knowledge about pesticide exposure via treated soil to non-target organisms, particularly vertebrates, is relatively sparse. Reptiles often nest within agricultural fields and thus developing embryos can be potentially exposed to pesticides. We tested the hypothesis that snapping turtle eggs (*Chelydra serpentina*) are impervious to absorption of pesticides, or alternatively that turtle eggs will absorb pesticides from treated soil. We exposed eggs to soil treated with a mixture of 21 pesticides, including herbicides, insecticides, and fungicides, at two concentrations (1.92 kg ha<sup>-1</sup> and 19.2 kg ha<sup>-1</sup> of a.i.). We sampled eggs after 1 day, 1, 2, and 3 weeks post-exposure, and measured residual pesticides in the egg contents. Pesticides were analyzed either using HPLC or GC-MSD. Seventeen out of 21 pesticides were detectable in eggs after 1 day of exposure. Pesticide burdens were highest at 3 weeks' exposure for the majority of herbicides, while many insecticides and fungicides peaked after 1 week. Generally, metribuzin, atrazine, and simazine had the highest absorption in eggs after 3 weeks at the 1.92 kg ha<sup>-1</sup> a.i. exposure (3.9, 2.7, 1.9 µg g<sup>-1</sup>, respectively). We used physical and chemical properties of the pesticides (e.g., Fugacity ratio, vapour pressure (Pa), water solubility (g (m<sup>3</sup>)<sup>-1</sup>), octanol-water partition and octanol-air partition coefficients (log K<sub>OW</sub> and K<sub>OA</sub>), Henry's

Law constant ( $\text{Pa} \cdot \text{m}^3 \text{mol}^{-1}$ ), organic carbon sorption partition coefficient ( $\log K_{oc}$ ), etc.) as predictors of absorption into the eggs.

### **Effects of triphenyltin exposure during the larval period in wood frogs (*Rana sylvatica*) (PO)**

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Triphenyltin (TPT) is a fungicide that is widely used in agriculture on crops such as pecans, potatoes and sugar beets. In areas of the United States, levels as high as  $6 \mu\text{g L}^{-1}$  TPT have been measured in the water of rivers and lakes. Furthermore, several studies have documented acute toxicity in some amphibian species at concentrations as low as  $1.25 \mu\text{g L}^{-1}$  TPT after 48 hours. However, to date no studies have been performed on the sensitivity of the wood frog (*Rana sylvatica*) to TPT. Thus, the current study assessed the sensitivity of wood frog tadpoles to TPT from 1 week post hatch through metamorphic climax. Wood frog tadpoles were exposed to 0.1, 1 and  $5 \mu\text{g L}^{-1}$  TPT. Endpoints to be examined include mortality, time to metamorphosis, basic morphometrics of tadpoles and metamorphs, and gonadal histology. Although this study and most analyses are ongoing, initial observations have shown that wood frog tadpoles suffer complete mortality after 9 d when exposed to  $5 \mu\text{g L}^{-1}$  TPT. During this same time period, mortality in control treatments was negligible. In conclusion, the wood frog is as sensitive to TPT as other amphibian species and 100% sub-chronic mortality was observed in concentrations that have been measured in water in the United States.

### **Morphometric and phenotypic effects of 17 $\alpha$ -ethynylestradiol exposure in the wood frog (*Rana sylvatica*) (PO)**

TOMPSETT, A. <sup>1</sup>, HIGLEY, E. <sup>1</sup>, WISEMAN, S. <sup>1</sup>, GIESY, J. <sup>1</sup> and HECKER, M. <sup>1</sup>. <sup>1</sup>University of Saskatchewan

Estrogenic chemicals in the environment have been implicated in causing a variety of adverse effects in exposed organisms. Exposure to estrogens, especially at critical times during sexual development, can cause feminization and/or demasculination of males of many amphibian species. 17 $\alpha$ -ethynylestradiol (EE2), a hormone analog commonly used in oral contraceptives, is an estrogenic chemical of particular environmental concern due to its great potency and ubiquitous release in sewage treatment plant effluents. In the current experiment, EE2 was used as a model compound in an exposure with wood frog (*Rana sylvatica*) larvae. The wood frog is native to temperate areas in North America and common throughout much of its large range. Wood frogs are sensitive to EE2 exposure during sexual differentiation and undergo male-to-female sex reversal when exposed at adequate concentrations. To cover the full spectrum of sex reversal, wood frog tadpoles were exposed to 1, 10, or  $100 \mu\text{g L}^{-1}$  EE2 from hatch through metamorphic climax. At metamorphic climax, weight and length of each animal were measured. In addition, gross morphology and histology of the gonads were examined. All analysis is ongoing.

## Effects of glyphosate formulations and polyethoxylated tallowamine on the survival and development of the wood frog (PO)

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Evidence is growing that glyphosate-based herbicide formulations are acting as endocrine disruptors and interfering with amphibian development. Polyethoxylated tallowamine (POEA) is a surfactant ("inert ingredient") included in some formulations that may have biological effects. Laboratory and mesocosm experiments were carried out as part of the LEWA.CA initiative to examine the effects of pesticides on the survival, development and sexual differentiation of wood frogs (*Lithobates sylvaticus*). Tadpoles were exposed to two 5-day pulses of treatment, initiated at Gosner stages (St.) 26 and 31, respectively. Treatment groups were: Control, WeatherMax (2.89 mg L<sup>-1</sup> a.e and 0.21 mg L<sup>-1</sup> a.e.), Vision (2.89 mg L<sup>-1</sup> a.e.), isopropylamine salt (2.89 mg L<sup>-1</sup> a.e.) and POEA (1.43 mg L<sup>-1</sup>). A chronic POEA (1.43 mg L<sup>-1</sup>) treatment was also applied to St. 26 tadpoles. Chronic POEA exposure caused high mortality. The effects of these compounds on development and sex ratios are under study. These results are the inception of a larger initiative to link developmental effects to changes in the expression of key genes controlling development. Supported by NSERC.

## Municipal Wastewater Effluent

### Overview of the ecotoxicity of urban effluents in aquatic ecosystems

GAGNÉ, F. <sup>1</sup>. <sup>1</sup>Environment Canada

Municipal effluents (ME) represent a major source of contamination to aquatic ecosystems. In addition to their physical characteristics, these effluents are highly complex mixtures of contaminants of biological and chemical origins. Moreover, ME represent a source for "new" emerging contaminants such as endocrine disrupters, pharmaceutical and personal care products, products derived of biotechnology (transgenes, proteins and microbes) and products from nanotechnology. The purpose of this study was to produce an overview of current and emerging environmental threats of ME to aquatic ecosystem. An emphasis was given to 12 years of active research within our research program at Montréal. The research priorities were given in respect to the two pillars of ecotoxicology—i.e., bioassay development and biomarker research. While the former will provide information on the relative toxic potency of the various substances and its mixture on organisms within food chains (multitrophic assays), the latter will provide tools to measure the toxic effects directly in the receiving water bodies in the context of cumulative effects of multiple stressors. Topics ranging from xenobiotic bioreactivity and biotransformation, endocrine disruption, inflammation and neuroexcitotoxic syndromes, the interaction of temperature (climate change) with ME contamination and disease resistance will be discussed. Recent developments on toxicogenomic-based biomarkers on emerging issues such as nanotechnology will also be discussed in the context of ME management and regulatory acceptance.



## **Intersex, reproductive impairment and fish community responses to multiple municipal wastewater discharges in the Grand River, Ontario**

TETREAULT, G. <sup>1</sup>, SERVOS, M. <sup>1</sup>, BROWN, C. <sup>1</sup>, BENNETT, C. <sup>2</sup> and MCMASTER, M. <sup>2</sup>.

<sup>1</sup>University of Waterloo, <sup>2</sup>Environment Canada

The Grand River watershed in Ontario receives the outflow of 29 municipal wastewater effluent (MWE) discharges, which is a mixture of domestic and industrial wastes, pharmaceuticals and personal care products (PPCPs). The purpose of this study is to develop a monitoring program with the capacity to evaluate the potential impacts of these discharges on fish and fish populations in the Canadian aquatic receiving environment. In 2005 and 2007, field studies upstream and downstream of two municipal discharges assessed fish communities (diversity and abundance), populations (length-frequency distribution and growth rates) and individual responses in terms of growth (condition factor) and reproduction (in vitro sex steroid production, gonadosomatic indices, and histology). Fish [Greenside darter (*Etheostoma blennioides*) and Rainbow darter (*Etheostoma caeruleum*)] collected downstream of the Kitchener and Waterloo municipal wastewater plants greater condition when compared to reference fish collections. This could be a reflection of the increased diversity and abundance of the benthic invertebrate community observed downstream of the discharges. Although fish populations did not display effects to MWE exposure, individual exposed fish demonstrated physiological alterations in sex steroid productive capacity and male fish demonstrated alterations in histopathology (intersex). Fish community assessments in 2007 and 2008 demonstrated significant alterations in fish abundance, diversity, presence of sensitive species, and evenness in the composition of the river fauna downstream of the MWE discharges.

## **Use of stable isotopes to trace municipal waste water effluent inputs through aquatic food webs within a highly developed river system**

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Previous studies have shown that the fate of municipal waste water effluents (MWEs) within the receiving environment can be tracked by monitoring the stable isotope signatures of resident aquatic organisms. In the Grand River watershed in southern Ontario, the stable isotope responses of resident aquatic organisms to the input from three MWEs of varying qualities were evaluated. It was observed that spatial and temporal variation in results limited our interpretation of results and led us to explore the mechanisms responsible for these changes in stable isotope signatures of resident aquatic organisms exposed to MWEs. Based upon our findings, four considerations regarding the use of stable isotope analysis to track the fate of MWEs are proposed as follows. Stable isotope signatures are 1) temporally and 2) spatially more variable in waste water exposed sites. 3) Stable isotope signatures of aquatic biota can be influenced by a river's larger biogeochemical cycle. Consequently, 4) interpretations of spatial and temporal isotopic trends in aquatic food webs are contingent upon an understanding of the biogeochemical response of rivers to individual MWEs. This work highlights the complexity of applying stable isotope analysis to track exposure of native biota to municipal waste waters and the factors which researchers interested in its application should consider when designing studies.



## Evaluating the efficacy of municipal sewage treatment technologies to reduce chemical contaminants and effluent ecotoxicity using screening, short- and long-term tests

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<sup>1</sup>Ontario Ministry of the Environment, <sup>2</sup>Environment Canada

As the first component of a larger research initiative, a baseline study was conducted to evaluate the reduction of effluent contaminant concentrations and aquatic toxicity using *in vitro* screening, and short- and long-term ecotoxicity tests on the effluent from two Ontario sewage treatment plants (STPs) operating as nitrifying activated sludge systems. Influent and effluent from both STPs were analyzed for conventional wastewater parameters as well as for a suite of "legacy" and "emerging" contaminants. Metals, pharmaceuticals, natural and synthetic hormones, bisphenol A, alkylethoxylates, and halohydrocarbons were measured in the final effluents of both STPs. PAHs were also measured in the effluent of one of the STPs.

Aquatic toxicity of the same effluents was evaluated using 24-h rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna* acute tests, 21-d *Ceriodaphnia magna*, 7-d fathead minnow (*Pimephales promelas*), 7-d *Lemna minor* and 96-h *Pseudokirchneriella subcapitata* sublethal tests as well as *in vitro* screening assays for endocrine-disrupting activity (yeast estrogenic screening assay (YES), yeast androgenic screening assay (YAS), and thyroid transport receptor (T4/hTTR) binding assay). The tests were conducted in triplicate. No short- or longer-term ecotoxicity was observed nor was there any indication that the effluents had potential to induce thyroid dysfunction. In contrast, results from the YES and YAS assays indicated that the effluents exhibited estrogenic and androgenic activity.

The next phase of this study will be to compare, at a pilot scale, the efficacy of different wastewater treatment technologies in reducing effluent contaminant concentrations and ecotoxicity using the same suite of screening, short- and longer-term tests.

## Fathead minnow lifecycle exposure to three Canadian municipal wastewater effluents

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<sup>2</sup>, SETO, P. <sup>1</sup> and BACKUS, S. <sup>1</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>Ontario Ministry of the Environment

Fathead minnows were exposed for a lifecycle to secondary treated municipal wastewater effluents (MWWEs) from three cities in Southern Ontario, Canada. The MWWEs (1,500 L) were collected weekly and fish were exposed to 70-100% effluent in flow-through aquaria in the lab. Exposures began at the fertilized egg stage, and continued through hatching, maturation and breeding. Fish were sampled at 5 months of age, and growth, health and reproductive status were assessed. Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) detected in the MWWEs included in descending order of concentration: trimethoprim, carbamazepine, bisphenol A, sulfamethoxazole, diclofenac, monensin sodium, ciprofloxacin, norfloxacin, clofibric acid and bezafibrate (at 560 to 140 ng L<sup>-1</sup>) and naproxen, gemfibrozil and ketoprofen (at 57 to 32 ng L<sup>-1</sup>). Fish grew well in all three effluents, but reproduction was decreased in two of the three effluents. The long term effluent exposures show the complex response of fish to the MWWEs, with normal growth, but decreased reproductive output. Studies will continue in

2010 to assess if model-scale advanced effluent treatment (increased nitrification and biological nutrient removal) will decrease PPCPs and the negative effects on fish egg production.

**To stabilize or not to stabilize . . . that is the question! Understanding pH stabilization during acute lethality testing within the new Wastewater Systems Effluent Regulation (WSER)**

*TAYLOR, L. <sup>1</sup> and SCROGGINS, R. <sup>1</sup>. <sup>1</sup>Environment Canada*

The proposed Wastewater Systems Effluent Regulations (WSER) were published in Canada Gazette 1 on March 20, 2010. The proposed regulations have been developed under the Fisheries Act and set national effluent quality standards for identified deleterious substances (i.e., BOD, SS, TRC and unionized ammonia) in effluent deposited by wastewater systems. They also define conditions to deposit effluent containing deleterious substances (for example, the effluent must not be acutely lethal). Systems must test for acute lethality with rainbow trout (either quarterly or monthly) using 1) Reference Method EPS 1/RM/13 or 2) EPS 1/RM/13 and EPS 1/RM/50. The first method refers to the standard 96-h single concentration (i.e., pass/fail) or multi-concentration test (i.e., LC50), whereas the second refers to an "add-on procedure" for pH stabilization which is done in conjunction with the standard 96-h trout test. This procedure was standardized to be used in the context of this national regulation with the objective to better account for toxicity due to ammonia, a pH dependant toxicant in municipal wastewater effluent. Understanding who can use pH stabilization, when the procedure can be applied, how it is performed, and why it is applicable to wastewater effluent will all be discussed. As the final WSER had not been published in Canada Gazette 2 at the time of abstract submission, we may need to revise this presentation closer to the conference date.

**Investigating the contribution of ammonia to the reproductive toxicity of sewage effluent in zebrafish (*Danio rerio*) reproduction**

*ALKEMA, K. <sup>1</sup> and VAN DER KRAAK, G. <sup>2</sup>. <sup>1</sup>University of Guelph, <sup>2</sup>Integrative Biology*

Recent research on final sewage effluent has focused on the negative impacts on fish reproduction of natural and synthetic hormones and pharmaceuticals that escape the sewage treatment process. Ammonia is also a common constituent of final sewage effluent and despite the widespread research on it, surprisingly little is known of its effects on reproduction in fish or its possible interactive effects with endocrine disrupting chemicals present in sewage. This research examined the effects of ammonia on reproduction in sexually mature zebrafish to determine the threshold concentration of effects as well as the mechanism of action. The threshold for observing a decrease in the egg production of zebrafish was less than 5 mg Total Ammonia-Nitrogen L<sup>-1</sup> which is well below the concentrations that are lethal to zebrafish. Also, full-grown follicles harvested from the ovaries of zebrafish exposed to ammonia for 24 hours are compromised in their response to the maturation-inducing steroid 17 $\alpha$ ,20 $\beta$ -P. Further studies provide evidence that ammonia does not act directly on the gonads but further up the HPG axis. Collectively these studies suggest that ammonia may contribute to the reproductive toxicity of sewage effluents.

## **A municipal wastewater effluent toxicity investigation: The good, the bad, and the ugly**

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A toxicity investigation was conducted for a municipal wastewater effluent (MWWE) following a rainbow trout and *Daphnia magna* acute lethality test failure (>50% mortality in 100% sample). The MWWE originated from a conventional activated sludge wastewater treatment plant that receives wastewater from residential, industrial, commercial and institutional facilities. Although initial Phase I TIE treatments suggested un-ionized ammonia was a potential cause of rainbow trout mortality, further investigations implicated other contaminants as the primary cause of toxicity. Specifically, lab testing combined with on-site investigations indicated toxicity was coincident with the appearance of excessive foam in the aeration basins. Foaming chemicals (e.g., cleaning products) were not used in the treatment system, therefore, the source of foam was presumably from an upstream input. Separation and isolation techniques were conducted and showed the foam was not only acutely lethal to trout, but also inhibited microorganisms found in activated sludge. The foam tested positive for methylene blue activated substances (MBAS) activity, signifying the presence of anionic surfactants. Initial attempts characterize the specific anionic surfactants showing MBAS reactivity were unsuccessful and additional experimentation was not pursued as the effluent became non lethal. This presentation will provide an overview of the methodology used to characterize toxicity of MWWE samples, and will describe the limitations and challenges faced by operators in resolving final effluent toxicity issues when treatment plants are challenged by upstream inputs that are difficult to predict or control.

## **Determining large-scale risk using available data: Receiving water risk assessment for municipal WWTPs in Alberta**

PRATHER, C. <sup>1</sup> and HUTCHINSON, N. <sup>2</sup>. <sup>1</sup>AECOM, <sup>2</sup>Hutchinson Environmental Sciences Ltd.

In 2008, Alberta Environment undertook a risk assessment of every WWTP in the province. It included an engineering (plant infrastructure/operating practices) and an environmental component based on near field and cumulative risk posed to surface water receivers by 325 surface water dischargers. The major challenge was to develop meaningful and repeatable methods for consistent risk assessment with the recognition that useful site specific receiving water data and effluent quality were only available for a few major dischargers. Sixteen risk metrics were developed that used available data to estimate site-specific and cumulative risks for each discharger on the basis of comparison of annual effluent loads to annual receiver loads for key conventional effluent parameters (phosphorus, ammonia, nitrate and oxygen demand). Receiver flow was the most important risk factor and was estimated for each site and for key reaches of rivers (for cumulative effects analyses) from Water Survey of Canada long-term flow records at 73 locations in the province. Long-term water quality data were available for 29 Alberta Environment sites and short-term or limited data from 90 Alberta Environment sites. This desktop assessment was favourably validated for a series of sites where there were detailed measurements of environmental responses to discharges. The results produced a series of recommendations for plant upgrades, monitoring and record-keeping programs.

### **Effects monitoring downstream of the Ottawa's ROPEC facility**

KILGOUR, B. <sup>1</sup> and BEZAIRE, B. <sup>2</sup>. <sup>1</sup>*Kilgour & Associates Ltd.*, <sup>2</sup>*City of Ottawa*

The City of Ottawa discharges upwards of 450 ML day<sup>-1</sup> of final treated municipal effluent from its R.O. Pickard Environmental Centre. The City adopted an environmental effects monitoring (EEM) approach to evaluating the nature, extent and cause of ecological effects based on a methodology for EEM developed by Environment Canada. Fish communities downstream of the final discharge are diverse and include sturgeon, walleye, channel catfish, and a variety of other species. The benthic invertebrate community in the near-field environment within 300 to 400 m of the diffuser has lacked sphaeriid clams and EPT taxa. Final effluent has been non-acutely lethal to trout and *Daphnia magna*. Final effluent has not produced any sublethal responses in fathead minnow or algae. Waterborne concentrations of chlorine and unionized ammonia have been at or above concentrations that are considered to pose risks to aquatic organisms. The results to date suggest ecological effects are localized and modest. The City has plans to add dechlorination to the final effluent process. Future EEM studies are in a good position to determine the ecological benefit of that action.

### **Municipal innovation in sustainable wastewater treatment: Stephenville's combined sewer/wetlands treatment systems**

SHARP, G. <sup>1</sup>. <sup>1</sup>*Abydoz Environmental Inc.*

Municipal sewage treatment using Kickuth Engineered Wetland technology has been commissioned during the fall of 2009 and the spring of 2010 for the town of Stephenville on the west coast of the island of Newfoundland using the "Root Zone" method developed in Germany. This is the largest subsurface wetland system (20,000 m<sup>2</sup>) providing secondary treatment in Atlantic Canada, servicing a population equivalent (P.E.) of 7,800 with an average daily flow of 4,555 m<sup>3</sup> day<sup>-1</sup>.

This presentation provides a brief history of the technology transfer, the pilots installed and their treatment results. Stephenville's combined sewer/wetlands treatment system is reviewed in detail, with a review of the design loadings and the overall sizing and components. The operation and overall performance will be reviewed with a summary of the monthly testing and treatment results that have been achieved since the system has been in operation.

This presentation discusses how the design of the wetland system accomplishes treatment of all the flow without a bypass system and protects the wetland from the push through created by the large infiltration of storm water. Also the concept of full treatment will be presented, where not only the effluent is treated by wetlands but the sludge that is removed in primary treatment is biodegraded in wetland sludge cells.

### **Wastewater monitoring under Canada's Chemicals Management Plan**

SMYTH, S. <sup>1</sup>, BARCLAY, K. <sup>1</sup> and NG, T. <sup>1</sup>. <sup>1</sup>*Environment Canada*

On December 8, 2006, the Government of Canada unveiled the Chemicals Management Plan (CMP) which takes action regarding chemicals that are harmful to human health or the environment. One element of the CMP is the collection of information on the properties and



uses of chemical substances that have been identified through the categorization process as high priorities for action. This information will be used to make decisions regarding the best approach to protect Canadians and their environment from risks these substances might pose. The wastewater sector has been identified as a possible release point to the environment for certain CMP substances. The activities associated with the CMP for the wastewater sector will include monitoring at different points of wastewater treatment systems including raw influent, final effluent, raw sludge and treated biosolids. Twenty wastewater systems across Canada, representing typical treatment processes including lagoons, enhanced primary treatment, and biological treatment, have agreed to participate in a wastewater monitoring program. The purpose of this program is to gather information to determine the levels of selected chemical substances entering wastewater treatment plants, the fate of these substances through the liquid and solids treatment processes at warm and cold process temperatures, and levels of these substances being discharged in wastewater treatment plant effluents and solids residuals. The results from this program will be used to improve our understanding and prediction of the fate of chemicals in wastewater treatment processes and to determine if control measures are needed to prevent these substances from entering the municipal wastewater system.

#### **Effects of municipal wastewater effluent on fish communities and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures in the Speed River, Ontario (PO)**

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There is concern that, with rapid urbanization, municipal wastewater effluents may degrade aquatic ecosystems unless larger investments in infrastructure are made. The City of Guelph, Ontario, is a municipality with an increasing population discharging a high-quality effluent into a low flow receiving environment. To support future management decisions, fish communities were evaluated by backpack electroshocking at nine sites in two seasons of 2008, and 3 seasons of 2009. This data will assist in determining current river conditions and the health of the fish community in the receiving environment. There were no large habitat differences among sites. In 2008, there was a significant decrease in Greenside darter (*Etheostoma blennioides*) and increase in Rainbow darter (*E. caeruleum*) abundances observed directly downstream of the outfall. Stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) also increased in Rainbow darter downstream of the outfall, but showed no change in Greenside darter. Darter abundances and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in August 2009 showed similar trends. It is possible the Rainbow darter immediately downstream of the outfall were exploiting a food source that was not present at upstream sites, resulting in a competitive advantage over the Greenside darter. Stomach content analysis was completed in each season of 2009 to help explain variations in isotopic signatures seen between darter species. Data from all sample times indicated seasonal differences in diet and isotope signatures. MWWs are complex and even tertiary treated effluent appears to have subtle effects on fish communities in small aquatic receiving environments.



**Characterization of commercial microbial products by polymorphic DNA markers and enzyme activity diversity: Occurrence and potential effects on freshwater mussels exposed to municipal effluents (PO)**

DOUVILLE, M. <sup>1</sup>, GAGNÉ, F. <sup>1</sup>, ZHU, B. <sup>1</sup>, FORTIER, M. <sup>2</sup> and FOURNIER, M. <sup>2</sup>. <sup>1</sup>Environment Canada, <sup>2</sup>INRS-IAF

The increasing commercial application of microbial products and consortia has raised concerns about their release to the environment and the potential for toxic effects on aquatic biota. This study characterized 25 bacteria, 3 fungi and 1 yeast using a combination of random amplified polymorphic DNA and 19 hydrolytic enzymatic activities (API ZYM), with the purpose of determining their occurrence and potential effects on the immune systems of mussels exposed to a primary-treated municipal effluent for 3 weeks. The 550 bp amplification product of *cpn60* gene by using universal primers was sequenced for each microorganism and available in GenBank. The microbial community structure in the digestive gland of mussels was also characterized using 16s ribosomal gene analysis by denaturing gradient gel electrophoresis (DGGE). The results revealed that each microorganism was easily cultivated with usual growth media and the enzymatic activity profiles permitted to propose an identification method using hierarchical tree and backpropagation neural networks classifications. The bacterial community structure based on DGGE of 16S rDNA in mussels changed with the concentration of the municipal effluent. This was also revealed by a characteristic change in enzymatic activity profiles with increased frequency in acid and amide phosphatases, leucine aminopeptidase and  $\beta$ -galactosidase in the isolated heterotrophic colonies in mussels. The immune system of the mussels was also compromised with decreased hemocyte density, increased phagocytosis and cytotoxic activities. While no trend between phagocytosis activity and the enzymatic activity profiles was found, the increase frequency in leucine aminopeptidase and acid phosphatase was negatively correlated with the hemocyte density and cytotoxic activity. In conclusion, a methodology to track for the occurrence, bioavailability and potential immunotoxic effects of commercial microorganisms is proposed based on a combination of genetic and phenotypic (enzymatic activity) markers and immunocompetence assessment.

**High levels of intersex found in two species of darter downstream of municipal waste water effluent (PO)**

TANNA, R. <sup>1</sup>, OAKES, K. <sup>1</sup>, MCMASTER, M. <sup>2</sup>, TETREALT, G. <sup>2</sup>, BENNETT, C. <sup>2</sup> and SERVOS, M. <sup>1</sup>. <sup>1</sup>University of Waterloo, <sup>2</sup>Environment Canada

The Grand River watershed in southern Ontario, Canada, is projected to experience population increases of 57% by 2031 with the majority of growth concentrated in urban centers (such as Kitchener – Waterloo, Guelph and Brantford) in the central portion of the watershed. The 26 wastewater treatment facilities in this watershed represent the largest point source discharges to the Grand River, with many of the effluents released having constituents capable of producing adverse effects in exposed fish. Intersex condition, where oocytes are found interspersed amongst the testicular tissues of male fish, represents a relevant whole organism biomarker of estrogenicity. To evaluate the extent and variability of intersex condition, a survey of 16 sites in the watershed was conducted by collecting resident Rainbow darter (*Etheostoma caeruleum*) and Greenside darter (*Etheostoma blennioides*).

Male gonad samples were evaluated for the presence of primary oocytes using a new quantitative evaluation technique. Sampling conducted in the fall of 2009 as well as the spring of 2010 indicated an increase in the occurrence of intersex condition in resident darters at some sites receiving wastewater effluent. Increasingly higher levels of intersex were found at sites downstream from multiple waste water plants.

#### **Fish communities to individuals: Weighing the evidence for environmental assessments for municipal wastewater (PO)**

*TETREAULT, G. <sup>1</sup>, SERVOS, M. <sup>1</sup>, BROWN, C. <sup>1</sup>, BENNETT, C. <sup>2</sup> and MCMASTER, M. <sup>2</sup>.*

*<sup>1</sup>University of Waterloo, <sup>2</sup>Environment Canada*

Municipal Wastewater Effluent (MWWE) is the largest anthropogenic discharge into waterways in Canada, which can lead to eutrophication, acute toxicity, and endocrine disruption. The Canadian government has proposed Wastewater Systems Effluent Regulations under the Fisheries Act to establish national effluent quality standards. The objective of the proposed standard is to reduce the risks to ecosystem health, fisheries resources and human health by decreasing the level of harmful substances deposited to surface water from wastewater effluent. Proposed regulations for MWWE include biochemical oxygen demand (BOD) ( $\leq 25 \text{ mg L}^{-1}$ ), suspended solids (SS) ( $\leq 25 \text{ mg L}^{-1}$ ), total residual chlorine ( $\leq 0.02 \text{ mg L}^{-1}$ ), and un-ionized ammonia ( $\text{NH}_3$ ) ( $< 1.25 \text{ mg L}^{-1}$ ). In order to evaluate the effectiveness of the national effluent quality standards in protecting fish and fish habitat, some municipalities will be required to conduct environmental effects monitoring studies. The effects on fish criteria have been set for the indicator of fish condition  $\geq 10\%$  of the reference area mean, and  $\geq 25\%$  for other indicators such as gonado- and liver-somatic indices. Since 2005 we have been studying the effects of fish exposed to MWWEs exceeding the proposed guidelines at three levels of organization (individuals, populations, community). Whole organism responses in wild fish have been observed in terms of condition, somatic indices (hepatic, gonad) and as well as some biochemical indicators and histopathology (intersex). Fish communities exposed to MWWE are also different than those from reference sites. We will discuss whether the data collected to date indicates if the proposed regulations will be protective of the aquatic receiving environment at these sites.

#### **Using chitobiase activity as an indicator of effluent impacts on secondary productivity: The Dead Horse Creek, Manitoba (PO)**

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Chitobiase is one of two moulting enzymes used to cleave chitin polymers that comprise the exoskeleton of aquatic arthropods and its rate of production has been used as an estimate of secondary production in zooplankton. During moulting this enzyme is released into the water and can be quantified by a simple fluorescence assay. Currently, the most common method of determining effluent impact on aquatic macroinvertebrate biomass in streams and rivers is through a benthic survey. This is costly, time consuming and can require extensive expertise. The rapid quantification of chitobiase activity and its rate of production could be used as a surrogate to estimate arthropod biomass in an aquatic system. Changes in chitobiase measures may identify areas in lotic systems where these organisms are being negatively impacted.

The Dead Horse Creek (in southern Manitoba) has three communities that release treated municipal sewage effluent into it at various points and times. This watershed is being used as a model system to assess the utility of chitobiase activity and production as an indicator of impacts on benthos. Samples for chitobiase and benthos were taken before and after effluent release throughout the watershed. We predicted that reaches of the creek experiencing effluent exposure would display reductions in the monitored chitobiase metrics that reflect impacts on the benthic communities. We were able to show that chitobiase production rates changed through the season, but zooplankton in the sewage lagoons added chitobiase to the effluent that masked any potential changes in benthic arthropod production rates. Further studies will be done to sort out the chitobiase in the effluent from the stream and how long water samples can be stored while maintaining chitobiase activity.

## *Toxicity of Metals*

### **Factors affecting mercury bioaccumulation in lake food webs of Kejimikujik National Park, Nova Scotia**

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Kejimikujik National Park, Nova Scotia, is recognized as a hotspot of mercury (Hg) contamination, with the highest known concentrations of this contaminant in the common loon (*Gavia immer*) of anywhere in North America. A recent study found that: 1) levels of Hg in Kejimikujik yellow perch (*Perca flavescens*), the primary prey of loons, increased by an average of 29% in ten lakes between 1996 and 2006; and 2) that methyl Hg (MeHg) concentrations in predatory invertebrates were the strongest predictors of total Hg (THg) in perch across lakes. It is unclear why levels of Hg in yellow perch are increasing in Kejimikujik, since decreases have been observed elsewhere due to stricter industrial emission controls. In 2009 and 2010, we conducted food web studies on seven systems in Kejimikujik representing a range of chemical characteristics. We are measuring Hg in food web organisms, sediments and water sources of energy (sulphur and carbon isotopes) to and trophic position (nitrogen isotopes) of the biota, and chemical characteristics of the lakes. Our aims are: 1) to better understand the structure of and energy sources to the food webs; and 2) to determine which factors account for the anomalously high levels of mercury in Kejimikujik perch. Preliminary results show that mean THg concentrations in perch range from 0.27 to 0.47  $\mu\text{g g}^{-1}$  (wet weight) across lakes; these concentrations seem particularly dependent on MeHg in dragonflies, as well as on dissolved organic carbon and iron in lake water.

### **Metal-gill binding and toxicity in fish: The influence of NOM quality**

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Natural organic matter (NOM) protects fish from metal toxicity through the formation of metal-NOM ligands, which decreases metal bioavailability. While predictive models of metal toxicity (e.g. Biotic Ligand Model) incorporate NOM quantity in toxicity predictions,

differences in the chemical composition (quality) of NOM are not presently considered. The goal of our work was to determine how NOM quality influenced metal-gill binding in rainbow trout (*Oncorhynchus mykiss*) exposed to Cu, Pb, Cd, or Pb plus Cd mixtures. Accordingly, trout were exposed to metals in the presence of (i) darkly coloured, highly terrigenous (allochthonous) NOM, (ii) mixed autochthonous-terrigenous NOM, or (iii) lightly coloured autochthonous NOM. The presence of NOM ( $4 \text{ mg L}^{-1}$ ) reduced Cu-gill binding by 70-90% in Cu-exposed trout, but each type of NOM reduced Cu-gill binding by an equivalent amount. In contrast, terrigenous NOM and mixed autochthonous-terrigenous NOM was much more effective at lowering Pb-gill binding in fish exposed to Pb than was autochthonous NOM. Cd-gill binding was also reduced in fish exposed to Cd, but Cd-gill accumulation increased in the presence NOM when fish were exposed to Pb plus Cd mixtures. We conclude that the need to consider NOM quality in toxicity predictions depends upon the metal(s) of interest, the quantity of metal, and the presence of other metals that could confound predictions of metal-gill binding and toxicity.

#### **Ameliorative effect of natural organic matter (NOM) on metal toxicity to aquatic organisms: Evaluating the influence of NOM quality**

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To evaluate the role of natural organic matter (NOM) quality, copper toxicity to *Daphnia magna* was investigated in the presence of seven different NOM isolates, previously-characterized using absorbance and fluorescence spectroscopy. Properties included absorbance ratios ( $E_{254}/E_{365}$  and  $E_{465}/E_{665}$ ), specific absorption coefficient ( $SAC_{340}$ ), fluorescence index (FI) and specific fluorescence coefficient ( $SFC_{420}$ ). In addition, fluorescent components (humic-like, fulvic-like, tryptophan-like, and tyrosine-like) of the isolates were quantified by parallel factor analysis (PARAFAC). At  $3 \text{ mg L}^{-1}$  dissolved organic carbon (DOC) added to moderately hard, dechlorinated Hamilton water ( $140 \text{ mg L}^{-1} \text{ CaCO}_3$  hardness), the average lethal concentrations (LC50s) of total Cu ranged from  $17.28 \pm 3.34$  (no NOM added) to  $116.34 \pm 33.35 \text{ } \mu\text{g L}^{-1}$  (marsh-NOM) with dissolved Cu accounting for on average of  $\geq 94\%$  of total Cu. At marginal statistical significance,  $E_{254}/E_{365}$  ratio (a surrogate for NOM molecular weight) was negatively correlated with LC50 while a positive relationship was observed with  $E_{465}/E_{665}$  (index of the alphatic nature of NOM), suggesting that isolates with higher molecular weight provide better protection. Although FI and  $SFC_{420}$  ranged between 0.22 to 2.54, and 1.51 to 19.34 respectively, these two fluorescence quality parameters were not significantly correlated to protection against Cu toxicity. Highly-significant positive correlations were determined between  $SAC_{340}$  (index for NOM aromaticity) and amelioration, supporting previous observation that NOM aromaticity is the key characteristics controlling their role as detoxifying agents of metals. Similar strong and significant correlations were found between humic-like and fulvic-like contents of NOM (normalized to DOC) and LC50 values, showing that isolates enriched in humic-like fluorophores were more protective than those with a higher composition of fulvic like fluorophores. Neither tryptophan-like nor tyrosine-like fluorophores were related to LC50 values, illustrating insignificant contribution of proteinaceous materials in NOM isolates towards protection against metal toxicity. Various NOMs alleviated Cu toxicity differentially, and easily-obtained optical properties could be used as quality measures to distinguish protection efficiency of one NOM from the other (NSERC Discovery, MITHE-SRN).



### **Cation-metal interactions and their effects on four species of *Daphnia* in soft water**

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The biological recovery of Sudbury lakes has been slow and incomplete despite the reductions in metal concentrations in the water achieved over the past thirty years. *Daphniids* (Crustacea, Cladocera) are lacking in many lakes. Almost no assessments of metal toxicity in soft water exist for *Daphniid* species native to Canadian Shield Lakes. To determine if the current copper and nickel concentrations in the Sudbury lakes are affecting their recovery, five lakes ranging in metal contamination were selected to test the performance of four species of soft water *Daphniids* native to the Canadian Shield. Two fourteen-day bioassays were performed: one with water from the lakes and another with water from one of them with added calcium or/and sodium. Among the selected endpoints to evaluate the performance of the *Daphniids*, the digestive tract alterations in the different metal concentrations indicate lack of digestion of the ingested algae and/or lack of food intake. The laboratory bioassays showed that the presence of sodium and calcium increased the survival of the *Daphniids* at metal concentrations that were otherwise lethal. The findings suggest that sublethal effects are operating at digestive level, impairing the *Daphniids'* ability to cope with physiological demands and environmental stress, and that those effects are diminished by the sodium and calcium levels seen in the Sudbury region.

### **The role of competing anions on the uptake of two metal anions (As(V) and Se(VI)) by freshwater algae**

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Many studies have established the competitive nature between phosphate and arsenate, and sulphate and selenate with respect to uptake by microorganisms from ambient water/media. Nevertheless, strong empirical data over a large range of environmentally realistic concentrations is lacking and the role that the competing analogs play on the ecotoxicological significance of these contaminants of concern is not well understood. A study was completed using freshwater algae cultured in the presence of  $10 \mu\text{g L}^{-1}$  As(V) and varying phosphate concentrations. With the use of AEC-ICP-DRC-MS technology it was determined that the uptake of arsenic was indeed negatively correlated with the uptake of phosphorus, with the algal cells preferentially taking in phosphorus in comparison to arsenic at a 100:1 molar ratio. In addition, speciation analysis of the algal culture medium suggested two different intracellular metabolic pathways for arsenic, methylation and reduction, based on the As-uptake rate. A similar project was conducted to establish the relationship between ambient sulphate concentrations and the rate of selenium accumulation into freshwater algae. This study provides useful information on the accumulation of selenium into primary producers and aids in predictions of selenium transport in the food chain. The bioaccumulation and biotransformation results of these studies are useful for site-specific environmental assessments of arsenic, whose risk is generally associated with water concentration/speciation, and selenium, whose risk is generally associated with accumulation in biota.



## **Bioaccumulation and physiological effect of chronic sublethal Cd exposure in rainbow trout**

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The objective of this research was to understand the physiological effects and accumulation dynamics of Cd in rainbow trout (*Oncorhynchus mykiss*) during chronic (30 d) sublethal waterborne exposures. Exposure concentrations were either 7 or 18 nM (0.75 or 2.0  $\mu\text{g L}^{-1}$ ) and trials were done in both moderately hard (140  $\text{mg L}^{-1}$  as  $\text{CaCO}_3$ ) and moderately soft (50  $\text{mg L}^{-1}$  as  $\text{CaCO}_3$ ) water. Cd resulted in either no (control and 7 nM Cd) or low levels of mortality (18 nM) during the first 5 d of exposure and disruption of Ca balance was evident. Accumulation was assessed on a whole tissue as well as a subcellular basis in gills, liver and kidney. Tissues accumulated Cd in a time- and dose-dependent manner. Fish exposed in moderately soft water accumulated higher amounts of Cd in gills, and on a subcellular basis more Cd was bound to sensitive fractions in all tissues in comparison to fish exposed in moderately hard water. Early mortality and ion-loss may link to accumulation in sensitive fractions (particularly within the gills); however, results were equivocal as Cd concentrations in these fractions continued to increase during exposure. Induction of reactive oxygen species was evident during the first days of exposure and defence mechanisms (e.g. catalase activity) were engaged and remained active throughout the exposure. Cd content in the heat-stable protein fractions of tissues increased significantly during exposure. These studies provide insight into accumulation-effect relationships for Cd and contribute to understanding how bioaccumulation can be applied in risk assessment.

## **Accumulation of selenium and effects in rainbow trout and brook trout: Highlights from a chronic endpit lakes study**

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Aquatic organisms have different sensitivities to selenium (Se). Rainbow trout exhibit a greater rate of Se-induced teratogenesis than brook trout, but little is known about the relative sensitivities of other physiological systems. To compare the effect of Se on rainbow trout and brook trout, juvenile rainbow trout and brook trout were stocked into reference ( $\leq 2.5 \mu\text{g L}^{-1}$ ) and Se-contaminated (15-35  $\mu\text{g L}^{-1}$ ) end pit lakes. Over two years, fish were sampled in the spring and the fall. Selenium accumulation rates, energy reserves, growth, physiological stress response, and oxidative stress biomarkers were monitored. Selenium accumulation in both species was greater in the Se-contaminated lakes than in the reference lakes and there was no significant species difference in the whole-body:muscle Se regressions. Chronic Se exposure interacted with food availability in the lakes to decrease energy reserves (liver glycogen and muscle triglycerides) in both species and condition was the lowest in the Se-contaminated lake with a poor food supply. Selenium exposure did not impair cortisol secretion in rainbow trout or brook trout and liver lipid peroxidation levels increased with duration of exposure in both species. Liver tocopherol and retinol levels will also be discussed. Data from this study can be used in species-specific risk assessments for Se. Funding: NSERC-MITHE-SN, AI GSS, and ACA Biodiversity Grant.

## **Effects of Se-methionine on the reproductive and physiological status of rainbow trout**

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This project investigates the effect of maternally transferred selenium-methionine (Se-met) on reproductive and physiological endpoints in rainbow trout. Holm et. al (2005) reported that native rainbow trout are more susceptible to the teratogenic effects of Se than brook trout and suggested this was due to oxidative stress caused by selenium compounds creating superoxide radicals. To test this hypothesis, rainbow trout were fed diets of 0, 15, and 40  $\mu\text{g Se g}^{-1}$  (dw) for 5 months prior to spawning. Tissue and blood samples were taken from adult fish at spawning, while eggs were fertilized and placed in a vertical incubator or under 15 cm of gravel in experimental flumes, to assess deformities and swim-up success. Plasma testosterone in adults increased in all treatments at spawning, while plasma T3 decreased compared to pre-treatment controls. A decrease in plasma T4 and a dose-dependent decrease in cortisol were detected in adult fish. Liver LPO and GSH were higher in fish fed selenium enriched diets. Swim-up success was significantly lower in the high treatment, while the control and low were not different. Decreased swim-up success in the highest treatment can be attributed to the teratogenic effects of Se. Dietary exposures of brook trout are in progress to provide comparative data for the reproductive effects of selenium in two salmonid species for use in species-specific risk assessment of selenium. (Funded by MITHE-SN)

## **Cumulative impacts and identification of cause: Bioavailability and toxicity of metals/metalloids in sediments collected from across Canada as part of MITHE-SN**

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Sediment concentrations, bioaccumulation and toxicity of 35 metals and metalloids in sediments and waters collected from across Canada at MITHE-SN (Metals In The Human Environment Strategic Network) field sites were assessed. Detailed risk analysis of a core group of 11 of these metals/metalloids was conducted using one method with one organism (*H. azteca*). The bioaccumulation data was used for risk prediction using the metal effects addition model (MEAM) to calculate the cumulative impact of the metals/metalloids. The study provided the samples and data to "field test" the MEAM. Identification of cause was determined by ranking each of the 11 metals/metalloids based on their contribution to toxicity according to the MEAM, as well as comparison to critical body concentrations. The remaining 25 metals/metalloids not included in the MEAM were evaluated for their contamination and bioaccumulation levels.

Field validation of the MEAM using lakes of varying water and sediment characteristics supplied biotic uptake and accumulation data from natural mixtures of metals in sediment and water and enabled the prediction of toxicity to be compared to observed toxicity.

## Canadian water quality guidelines for cadmium, uranium and zinc

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Canadian Water Quality Guidelines (CWQGs) are developed under the auspices of the Water Quality Task Group of the Canadian Council of Ministers of the Environment (CCME). The National Guidelines and Standards office of Environment Canada is the federal member and technical secretariat to this task group. Following the CCME protocol (2007), CWQGs (both short-term and long-term) for cadmium (Cd), uranium (U) and zinc (Zn) were developed using a species sensitivity distribution (SSD). Each of these metals had a large toxicity dataset that was evaluated and screened according to standard criteria. The final sets for each contained from 150 to over 400 acceptable data points. For the long-term CWQGs, these points were further ranked from EC10, MATC, NOEC, EC20, LOEC, EC<50, to non-lethal EC50; only the lowest endpoint for each species was selected for the SSD. The SSDs for Cd, U, and Zn included 29, 13, and 33 aquatic species, respectively. Several model curves were evaluated and tested for goodness-of-fit. The Canadian Water Quality Guideline is set at the 5<sup>th</sup> percentile on the curve. The guidelines for cadmium and zinc can be adjusted based on local water hardness. The CWQG for Cd and Zn will be posted for public review this fall, while the CWQG for U is awaiting final approval by the CCME.

## Acute and chronic effects of bioaccumulated Cd on *Lumbriculus variegatus*, *Lymnaea stagnalis* and *Hyaella azteca*

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Objectives of this study were to understand accumulation-effect relationships for Cd in both Cd-resistant as well as Cd-sensitive organisms. Acute and chronic exposures were done with three organisms, *Lumbriculus variegatus* (resistant) *Lymnaea stagnalis* (intermediate sensitivity) and *Hyaella azteca* (sensitive). *Lumbriculus* survived at concentrations up to 1850 nM (200 ug L<sup>-1</sup>) and accumulated significant amounts of Cd but did not reach steady state during exposure (up to 3 months duration). Most of the accumulated Cd was stored in subcellular fractions representing detoxified metal. Chronic endpoints in *Lymnaea* were mortality, growth (shell length, weight), whole body ions and Cd bioaccumulation. Responses to Cd exposure varied with size, with smaller individuals being more sensitive. In 31 d static renewal tests with small organisms (5 mm shell length) in hard water (140 mg L<sup>-1</sup> CaCO<sub>3</sub>) concentrations >280 nM (30 ug L<sup>-1</sup>) resulted in 100 % mortality, and a LC50 value of 120 nM (13 ug L<sup>-1</sup>) was calculated. Growth was inhibited at exposure concentrations greater than of 92 nM (10 ug L<sup>-1</sup>) and this resulted from reduced food consumption. Similar exposures in soft water (40 mg L<sup>-1</sup> CaCO<sub>3</sub>) showed growth effects at 74 nM Cd (8 ug L<sup>-1</sup>). Effects on *Hyaella azteca* occurred at Cd concentrations as low as 18 nM (2 ug L<sup>-1</sup>), and while organisms reached steady state quickly, accumulation was variable. The use of accumulation in resistant organisms as a biomarker of effects in sensitive organisms will be characterized. This research is supported by the NSERC Strategic Program with Rio Tinto Alcan and Environment Canada.

## Development of a revised Canadian Water Quality Guideline for silver

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Data from a literature review on silver chemistry, bioaccumulation and toxicity and the recently revised Canadian Council of Ministers of the Environment (CCME) protocol for the derivation of environmental quality guidelines were used to derive revised water quality guidelines for silver. Silver generally occurs in the aquatic environment at concentrations in the range of 1-10 ng L<sup>-1</sup> and is often bound to particles and colloids or complexed by dissolved ligands. An extremely small fraction of the silver present in natural waters will actually occur as free silver ions. Complexation of silver-dissolved ligands has been associated with reduction of silver toxicity to freshwater and marine species, while competitive interactions with cations also reduce the toxicity of silver to freshwater species. The mechanisms of the acute toxicity of silver to freshwater fish are well understood and silver toxicity is associated with ion regulation disruption on the gill's Na uptake channels. Complexation of silver by natural organic matter, chloride, and other ligands containing sulfur has also been shown to markedly reduce the toxicity of silver. Bioaccumulation of silver is dependent on exposure concentrations and silver speciation and is not associated with physiological effects. There is no evidence of silver biomagnification and as observed for other trace metals, bioconcentration and bioaccumulation factors actually decrease with exposure concentrations. For both short- and long-term exposure in freshwater, sufficient data were available to meet the requirements of the CCME protocol to derive guidelines based on statistical distribution, and species sensitivity distributions were used for the freshwater guidelines. Data were also available to derive marine guidelines based on species sensitivity distributions.

## Hemimysis in Lake Ontario and its impact on mercury food web transfer

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*Hemimysis anomala* (the bloody red shrimp), an invasive invertebrate of Great Lakes, was first reported in Lake Ontario in 2006. Literature from their native range (Ponto-Caspian region of eastern Europe) suggests *Hemimysis* prefer nearshore, warmer waters and have rapid propagation and opportunistic omnivore feeding habits (zooplankton, phytoplankton, insect larvae, etc). To examine their influence on the food web dynamics of today's Lake Ontario, we sampled multiple trophic levels, from algae to piscivorous fish, across a gradient of *Hemimysis* density in the Canadian waters of Lake Ontario in spring, summer and fall of 2009, analyzing all food web components for mercury, stable nitrogen and carbon isotopes. Results showed that *Hemimysis* has distributed widely in Lake Ontario and their density could reach 200 individual/ m<sup>3</sup> in some places where full-scale aquatic food webs that include *Hemimysis* and important native species were figured out. The mercury concentration of *Hemimysis* from Waupoos and Cobourg varied from 11.9 ng g<sup>-1</sup> ww to 21.1 ng g<sup>-1</sup> ww, and very close to Hg concentration of round goby *Neogobius melanostomus* and alewife *Alosa pseudoharengus*, which are reported to prey on *Hemimysis* predominantly in southeastern Lake Ontario. Based on other mercury analysis results, our study suggested that small alewife preyed on *Hemimysis* with relatively few opportunities; Brown trout, lake trout, and other top



consumers have not been directly impacted by *Hemimysis* in Lake Ontario at present. However, *Hemimysis* do elevate the mercury biomagnification of food chains where they comprise one part of the food chain.

## ***Regional Monitoring Frameworks***

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### **An integrated framework for watershed cumulative effects assessment: The CWN watersheds consortium effort**

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There are a wide variety of aquatic monitoring programs that currently exist in any watershed, including pre-construction environmental impact assessments, compliance permit monitoring, environmental effects monitoring (EEM), cumulative effects assessment, crisis and spills management, and regional and national monitoring programs. The focus of the programs ranges from predictive to retrospective approaches, and each of these programs has developed their own monitoring strategies, study designs, methodologies and interpretation frameworks. Linking the programs in a unifying framework is complicated by the absence of a common philosophy, or standardized study designs for various programs operating in ecosystems, and the absence of an integrated approach. We have been working on an integrating framework for environmental monitoring to integrate environmental health assessment into a strategic framework that moves towards a watershed planning process. The move towards an integrated approach requires the development of a level of commitment to baseline monitoring, post-operational assessment, and an increasing role for science in decision making that currently does not exist in most jurisdictions.

### **The need for integrated regional monitoring frameworks to support watershed cumulative effects assessment: A case study for Saskatchewan**

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In Canada, as in many other parts of the world, we are hindered by our abilities to integrate information on watershed change and risk in an efficient, effective, and scientifically defensible manner. This hindrance has had consequences to both human and ecological health. Assessment and management of source water to ensure public and environmental protection must be integrated in a system-driven context; that is the fundamental basis of integrated water resource management. Unfortunately, implementing this rather simple idea has proven to be difficult. Our team at the University of Saskatchewan has developed a system-based integrated assessment approach for application at watershed scales that integrates water quality and quantity metrics to measure changes over broad temporal and spatial scales. This provides an understanding of where changes have occurred, the magnitude of the changes and the direction of the changes. All changes in parameters are evaluated against "reference" benchmarks determined from natural variability using a statistically rigorous and quantifiable approach. The THREATS effects-based approach



identifies "hotspots" and "hot moments" for assessing and managing Canadian watersheds towards sustainability and has been applied to large watersheds in Saskatchewan including the Quapelle River Basin and the South Saskatchewan River Basin. Most importantly the scientific change assessments are reported in a risk-based context and made available through our THREATS software as a legacy instrument for ongoing assessment, adaptive management, knowledge translation and education. The approach will be extended to other Canadian watersheds as well as global watersheds. Our success has been founded in understanding that irrespective of the watershed examined, core commonalities in water quality and quantity assessment exist and must be built into a decision-making framework for consistent application over time within a watershed and across different watersheds. It is only in this manner that a scientifically rigorous process can be consistently developed over the temporal and spatial scales required. We will illustrate how these change assessments can be linked to causal identification, adaptive management frameworks and watershed management plans.

### **The "power" of regional data: Water quality monitoring in the lower Athabasca basin using a reference-condition approach**

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Since its establishment in 1997, the Regional Aquatics Monitoring Program (RAMP) has collected nearly 80,000 water quality observations from numerous waterbodies throughout the lower Athabasca basin. A key objective of the RAMP water quality component is to detect and assess environmental change, which has been defined in this case as water quality outside the range of natural variability. Meeting this objective required development of an analytical approach that allowed for a robust definition of natural variability, and defensible, meaningful comparison of newly collected monitoring data against a defined range of regional baseline conditions. Analytical designs based on statistical power (i.e., management of Type II error in ANOVA-type comparisons) are of limited value for operational water quality monitoring programs, for numerous reasons. Instead, RAMP has developed a reference-condition-based approach to water quality analysis and assessment, which takes advantage of the very large, regional dataset being generated by the program. This approach allows multiple comparisons of water quality data (upstream/downstream, gradient, baseline/test, before/after, time-trend) against defined ranges of regional baseline conditions, and addresses many of the deficiencies of power-based analyses.

### **Developing a regional monitoring network for Northumberland Strait, southern Gulf of St. Lawrence**

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Northumberland Strait, separating Prince Edward Island from New Brunswick and Nova Scotia within the southern Gulf of St. Lawrence, has provided sustenance to Mi'kmaq, Acadian and British communities for centuries. Over the last two decades, however, local residents have become concerned about declining fisheries resources in the rivers, estuaries and Strait itself. Initially, the construction of Confederation Bridge (1993-1997) was blamed but it soon became clear that broader changes were occurring in the environment. Some of these changes were obviously related to anthropogenic activities on various spatial and

temporal scales, but others not. In 2005 the federal Minister of Fisheries and Oceans instituted a working group to address these concerns and in 2007 an Ecosystem Overview Report (EOR) was published. Recommendations leading from the EOR included research into the physical and biological oceanography of the Strait and its marine environmental quality. This research is being carried out by DFO through an Ecosystem Research Initiative funded from 2008-2012. Next steps need to include the watersheds with their rivers, estuaries and contiguous coastal environments which may be a major influence on the Strait. The Canadian Water Network provides an opportunity for this research through establishing communities of practice or consortia that focus on specific areas of joint interest and identified priorities. We propose to develop a regional monitoring network in support of cumulative effects assessment in the Northumberland Strait region, focusing on the basins draining into the Strait. In addition to addressing the original concerns over changing fisheries and environmental conditions, this project will provide an opportunity to carry out research into effects of pollutant loadings and climate change in an area projected to see major changes.

### **Fish community responses to municipal effluents in the Grand River watershed**

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The Grand River is a small watershed in southern Ontario that receives a number of stressors associated with intensive agriculture, municipal effluents and urbanization. Our work is exploring the impacts of stressors, including emerging contaminants of concern, associated with several municipal outfalls. The work includes the examination of specific chemicals by trace analysis (e.g. LC-MS/MS) and bioassay directed Toxicity Identification Evaluations (TIE) using several endpoints, including estrogenicity. Biological impacts on fish have been examined at several levels of organization, including gene expression, circulating sex steroids, and vitellogenin induction in cage fish. In addition, whole organism responses in wild fish have included assessment of condition, somatic indices (hepatic, gonad) and intersex. Fish communities have been surveyed at numerous sites in the shallow (riffle) environments across the watershed. Stable isotopes of carbon and nitrogen have shown changes in nutrient dynamics associated with the various outfalls. The work has shown a variety of changes in fish responses and communities in the watershed associated with municipal effluents although they differ by effluent, species and receiving environment across the watershed. The chemical exposure and biological responses will be presented in relationship to the characteristics of the wastewater treatment plant processes and the receiving environment.

### **Loadings of polynuclear aromatic compounds and metals to the Athabasca River watershed by oil sands mining and processing**

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The contribution of the oil sands industry to the pollution of the Athabasca River is highly controversial. Wastes include water, sand, silt, clay, bitumen, and contaminants such as polycyclic aromatic compounds (PAC) and metals. Upgrading bitumen to synthetic crude

requires coking and coke combustion, which release significant quantities of PAC and metals. In 2008, we measured PAC and metals in snow and river water of the Athabasca watershed and found that the industry is a significant source of contamination. Within 50 km of upgrading facilities, there was a strong signal of contamination by airborne particulates, PAC, and metals, equivalent to an annual loading of 600 T of bitumen. In addition, there was a strong association of contamination with proximity to land clearing, mining, road dust, or other stack emissions. The strongest signals were in summer, suggesting surface run-off of contaminated water, and contamination was related most strongly to recent development, between 2006 and 2008. Tributaries in watersheds with little or no development showed that oil-sands-related contamination was not due to natural erosion. In contrast, some river sites showed signs of contamination by process wastes from effluents or tailings ponds. Some metals were still elevated above background as far downstream as L. Athabasca. The observed concentrations of PAC in snowmelt and tributary waters suggest an increased risk of toxicity to embryos of spring-spawning fish. Airborne metals may also contribute to high concentrations of Hg and Cd in fish and wildlife consumed as country food. Major changes are clearly needed in the way that the environmental impacts of oil sands development are monitored and managed.

#### **Monitoring mercury in freshwater fish in the oil sands region of northern Alberta: Spatial and temporal comparisons to regional mercury concentrations in fish**

KEITH, H. <sup>1</sup>. <sup>1</sup>*Hatfield Consultants*

The Regional Aquatics Monitoring Program (RAMP) is an environmental monitoring program in the oil sands region of northern Alberta, Canada. The program is designed to fulfill the aquatic monitoring needs of all RAMP stakeholders, to understand the potential effects of oil sands development on aquatic systems, and to address specific issues important to communities of the region. Mercury concentrations in fish have become a growing concern for communities dependent on this resource for their livelihood. Potential mercury sources entering the aquatic systems within the oil sands region include muskeg dewatering, deforestation, flooding, and air emissions. To assess the suitability of fisheries resources for human consumption and the potential cumulative effects on fish health, RAMP annually collects non-lethal tissue samples for mercury analysis from northern pike (*Esox lucius*), walleye (*Sander vitreus*), and lake whitefish (*Coregonus clupeaformis*) in various rivers and lakes within the oil sands region. To provide a regional context to the RAMP monitoring results, a mercury database was developed from a synthesis of studies conducted in other regions of Alberta and across Canada between 1975 and 2009. All data points were mapped to assess spatial and temporal differences in mercury concentrations and any exceedances of subsistence ( $0.2 \text{ mg kg}^{-1}$ ) and general ( $0.5 \text{ mg kg}^{-1}$ ) consumption guidelines. By assessing the RAMP fish tissue monitoring results in relation to fish mercury concentrations in watercourses and waterbodies outside of the oil sands region, any changes in mercury concentrations in fish can be assessed as localized to a specific waterbody or as regional in nature.

## **Development of an effects-based assessment framework for monitoring of dredged material disposal sites**

VALOIS, A. <sup>1</sup>, MUNKITTRICK, K. <sup>1</sup> and MACDONALD, A. <sup>2</sup>. <sup>1</sup>Canadian Rivers Institute, <sup>2</sup>Environment Canada

Since the early 1950s, materials dredged from the Saint John Harbour (Saint John, New Brunswick), one of Canada's major ports, have been dumped at the open-water site near Black Point. Black Point was classified as a dispersive site at the time of designation, and although most of the material placed at the site has been dispersed, approximately 16% remains on-site. Significant toxicity to invertebrates has been found in sediments and there are concerns that disposal activities may be affecting commercially important lobster populations. Traditionally there has been an individualistic approach to monitoring at the Black Point Ocean Disposal Site and the lack of standardized methods has limited the application of the results, primarily due to an inability to share and compare data over time. Monitoring is necessary to determine the size of the disposal site footprint, if and where the disposal material is moving, and what environmental effects this movement (if any) is having on adjacent areas. The goal of this work is to develop a long-term, standardized effects-based assessment framework for monitoring at the Black Point Disposal Site to assess whether the ocean disposal regulations, guidelines and permit conditions are adequate in protecting the marine environment.

## **Developing a regional monitoring framework for the Saint John Harbour, NB**

ARCISZEWSKI, T. <sup>1</sup>, FARQUHARSON, S. <sup>1</sup> and MUNKITTRICK, K. <sup>2</sup>. <sup>1</sup>CRI, <sup>2</sup>University of New Brunswick

The city of Saint John is located at the mouth of the Saint John River on the Bay of Fundy. In addition to the estuary being the eventual recipient of the upstream wastes in the Saint John River basin, the harbour also has many industrial discharges, including a pulp & paper mill, an oil refinery, a tissue paper plant, a brewery, an oil-fired power plant, and many sewage outfalls (including untreated sewage). There are currently more than ten programs monitoring over 20 parameters (i.e., metals, effluent toxicity, benthic community) at greater than 15 sites in the harbour, under ten different regulatory requirements. Stakeholders have identified more than 25 anticipated future developments or concerns in the harbour area, including the potential for Saint John to be the energy hub of the east coast. We recognized that the harbour could benefit from a regional approach to monitoring by coordinating, standardizing, and harmonizing the various work in the harbour. We have been facilitating a process to enable opportunities to develop a regional monitoring framework and address cumulative effects. This talk will give an overview of the process we have used, including the development of a regional consortium and working group.

## **Monitoring of lower order streams in Uruguay (PO)**

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In Uruguay the watersheds are mainly covered by lower order streams, highly transformed by land cover changes. In particular, the expansion and intensification of



agriculture has brought transformation of their physical, chemical and/or biological characteristics. Changes in surface runoff, loading of nutrients, organic matter, pesticides and total solid suspended are some well documented effects. However, the effects on natural dynamics of biological communities are insufficiently known. Consequently, the aim of this work was to provide information on the integrity of lower order streams and to develop strategies that help in watershed management programs using attributes of small fish assemblages. Scientific interest in small fish assemblages has increased in recent years and has been included in national environmental monitoring programs such as those in the U.S and Canada. Environmental and fish samples were collected from agricultural and forested basins to assess the relationship between land cover and seasonal variation in diversity, richness, composition, abundance and distribution patterns of fish species.

### *Use of Whole Organisms*

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#### **Assessing impact of biosolids pellets to *Daphnia magna* bioassays**

BROSE, H. <sup>1</sup>, MCCARTHY, L. <sup>1</sup> and PUDDPHATT, K. <sup>1</sup>. <sup>1</sup>Ryerson University

Sewage treatment plant biosolids are the by-product of wastewater treatment and biosolid pellets are a form of heat-treated biosolids. Both biosolids and biosolid pellets contain valuable nutrients that can be applied to land for the benefit of plants and soil. But while biosolids (regulated provincially) have restrictions on their application, pellets are regulated federally by legislation with no monitoring requirement. While both biosolids and pellets contain beneficial compounds, they also have some components of concern (heavy metals, pathogens, and various organic contaminants). With no restrictions on their application, pellets are at a higher risk for coming into direct contact with biota in adjacent receiving waters. Additionally, an extensive literature review indicated that little research has been conducted to assess the potential impact of biosolids pellets on indigenous organisms. This study aimed to rectify this paucity of information by examining the potential ecological impact of biosolid pellets. Additionally, the limitations to the current assessments of microbial inactivation during pelletization found inside Toronto Public Health's 2004 "Biosolids Pellet Review Study" (the single publication dedicated to biosolid pellets) will be discussed. Preliminary bioassays, utilizing the freshwater zooplankton *Daphnia magna* in a series of acute, chronic, and reproductive tests, were conducted with the addition of pellets in a representative bioassay system. Results showed the formation of biota-containing floc in the presence of *Daphnia* and biosolid pellets and will be briefly discussed.



### **The toxicity of Harmony Landfill leachate to green hydra (*Hydra viridissima*)**

GINOU, C.<sup>1</sup>, GUCHARDI, J.<sup>1</sup>, ORREGO, R.<sup>1</sup> and HOLDWAY, D.<sup>1</sup>. <sup>1</sup>University of Ontario Institute of Technology

Harmony Landfill is a former industrial waste disposal site located adjacent to Harmony Creek in Oshawa, Ontario, Canada. The landfill was operated from 1957 until 1980, and during active disposal, approximately 1 million tonnes of waste were land-filled on a 9 hectare portion of the site. Although past environmental monitoring had indicated localized contamination of ground and surface waters by Harmony Landfill leachate, the present level of impact remained unclear. In order to determine the potential of Harmony Landfill leachate to affect aquatic organisms in Harmony Creek, chemical analysis of field samples and laboratory toxicity testing were performed. Chemical analysis was completed on field samples from Harmony Creek, upstream and downstream of the landfill, and with leachate samples collected seasonally at Harmony Landfill from December 2008 until April 2010. Toxicity tests were conducted using the model freshwater invertebrate Green Hydra (*Hydra viridissima*). Hydra were pulse-exposed for 24 hours to varying concentrations (0%, 3.2%, 10%, 32%, 100%) of field-collected leachate samples diluted with laboratory water, 100% upstream creek water and copper sulphate as a positive control. Population growth, Hydra morphology and survival were recorded daily for 7 days. Population growth was significantly inhibited compared to lab water (0%) controls at the 100% leachate concentration in December 2008 and July 2009. Hydra morphology (32% and 100%) and survival (100%) were only affected by the December 2008 leachate. Overall, Harmony Landfill leachate appears to have a low risk of negatively affecting aquatic invertebrate populations in Harmony Creek.

### **Effects of chronic exposure to the aqueous phase of produced water on growth, appetite and immune response of Atlantic cod**

PEREZ CASANOVA, J.<sup>1</sup>, HAMOUTENE, D.<sup>1</sup>, BURT, K.<sup>1</sup>, HOBBS, K.<sup>1</sup>, KENNY, S.<sup>1</sup>, LUSH, L.<sup>1</sup>, BUNGAY, T.<sup>1</sup>, MURRAY, H.<sup>1</sup>, INGS, D.<sup>1</sup> and LEE, K.<sup>1</sup>. <sup>1</sup>Fisheries and Oceans Canada

The biggest discharge from the offshore oil industry is produced water (PW). As new technologies emerge to remove the oil from such discharges, the question remains as to the effect that the water-soluble fraction of contaminants present in PW may have on the biota surrounding the areas of discharge. Furthermore, PW has been shown to exert its effects over larger areas from the oil platforms than previously thought. Thus, we investigated the effects of long-term (8 weeks) intermittent exposure (3 times per week) to environmentally relevant concentrations of the aqueous fraction of PW: 100 ppm (10000× dilution) or 1000 ppm (1000× dilution) on growth parameters, food consumption, innate immune response (respiratory burst activity of head kidney leukocytes: RB), and mRNA expression of appetite- and immune-related genes of Atlantic cod. No significant effects were observed in food consumption or growth parameters. The RB of head kidney leukocytes was significantly decreased in both the 100 and 1000 ppm groups when compared to the control group. Results indicate that chronic exposure to the aqueous fraction of PW seems to have a negative effect on the innate immune response of Atlantic cod. The results of mRNA expression studies will also be discussed.

## Investigation of whitefish health from northern Saskatchewan lakes

HURSKY, O. <sup>1</sup> and PIETROCK, M. <sup>1</sup>. <sup>1</sup>University of Saskatchewan

Over the past few years an increase in fish parasitism has been observed in many Indigenous communities in northern Saskatchewan, leading to concerns that changes in parasitism could be related to industrial activities in the North and environmental pollution. Therefore, a study has been initiated to investigate parasites and chemical contaminants in whitefish (*Coregonus clupeaformis*) from Montreal and Reindeer lakes to determine fish health and condition and to identify contamination levels. In 2008/2009 a total of 162 whitefish from both lakes were sampled and analysed for biological (parasites) and chemical (metals, PCBs) contaminants. A total of 20 species of parasites were found belonging to the taxa Cestoidea, Trematoda, Nematoda, Acanthocephala, Copepoda, Monogenea, and Hirudinea. Some of the parasites may have implications for fish health. In Montreal Lake, the most prevalent species was *Ichthyocotylurus erraticus* (Trematoda) found to parasitize heart of all examined fish. The most prevalent species in Reindeer Lake was *Diplostomum* sp. (Trematoda) found in the eye lenses of 87.9% of the fish. Condition calculations revealed satisfying condition of whitefish in both lakes. However, mean gross energy estimates suggest that the fish have low energy resources to withstand stressful conditions. Contamination of fish with metals and organic compounds was low. PCB congener concentrations from both lakes were extremely low, with many below the detection limit. In summary, health of whitefish from Montreal and Reindeer lakes is satisfying. There is no indication that environmental pollution may be causative for the observed modifications in fish parasitism.

## Toxicity identification and reduction evaluation of a former Kraft pulp mill landfill leachate

EICKHOFF, C. <sup>1</sup>, KEATING, J. <sup>1</sup>, STAVROFF, L. <sup>1</sup>, PICKARD, J. <sup>1</sup>, CHAN, C. <sup>2</sup>, JOHNS, R. <sup>2</sup>, PERELLO, F. <sup>2</sup> and KORMENDY, R. <sup>3</sup>. <sup>1</sup>Maxxam Analytics, <sup>2</sup>Keystone Environmental Ltd., <sup>3</sup>Western Forest Products Inc

Recurring toxicity was observed in a leachate from a former Kraft pulp mill landfill using the acute 96hr rainbow trout test. After the installation and construction of a treatment plant for the landfill, the composition of the leachate changed and the treatment technologies employed could not ensure removal of toxicity in the discharge samples. Contaminant levels in the leachate were compared with the BC freshwater quality criteria but no obvious contaminants were identified. The variability of the leachate and the potential for additional toxic constituents created an uncertainty in the treatment process. Therefore, a TIE was conducted to identify the toxic agents in the leachate. The objectives were to identify the toxic agent(s), to evaluate treatment strategies, and to pass the acute toxicity test. Phase I TIE results showed that toxicity was linked to pH, and that ammonia was present at concentrations that could result in toxic levels of unionized ammonia at ambient test temperatures and a pH greater than 8.6. Phase II and Phase III TIE results confirmed that observed toxicity in test samples was caused by unionized ammonia. Based on these results, operational changes and upgrades to the treatment systems were utilized to reduce the discharge toxicity. The work generated information regarding the composition and toxic components of leachate, the effectiveness of the leachate treatment technologies, and the identification of improved toxicity testing methods. While ammonia reduction was achieved

by the improved treatment technologies, the creation of a new toxicity testing procedure was the key to meeting the permit requirements for discharge.

### **Evaluation of the multispecies freshwater biomonitor to determine behavioural effects of tributyltin and atrazine on *Daphnia magna* and *Hyalella azteca***

FLEET, V.<sup>1</sup>, MCCARTHY, L.<sup>1</sup>, BOSTAN, V.<sup>1</sup> and LAURSEN, A.<sup>1</sup>. <sup>1</sup>Ryerson University

Early-warning biomonitoring systems (EWBS), when rigorously tested and assessed for scientific veracity, can provide continuous monitoring of changes in water quality. The Multispecies Freshwater Biomonitor (MFB) is supposedly just such a system, recording the behaviour of aquatic organisms using an electric field and detecting changes in the movements of biota which may be caused by stressors such as aquatic contaminants. In this study, the MFB was used in an extensive suite of experiments in an effort to detect behavioural changes of *Daphnia magna* and *Hyalella azteca* when exposed to tributyltin (TBT) and atrazine. The applicability of the MFB to be used as a monitor of drinking water quality as well as the usefulness of the organisms in this automated system was determined. While responses in behaviour were seen with the human eye, neither contaminant brought about behavioural changes in either organism that were detectable by the MFB, even at the highest tested concentrations. While extensive literature indicated that this system was indeed useful for field applications, this study concluded that the MFB is not yet ready for use in the field to detect contaminants entering a water system when using *D. magna* or *H. azteca* as test species. Future research is required for examination of other species' ability to detect aquatic contaminants and whether the MFB is able to detect such responses.

### **Assessing the usefulness of the automated behavioural monitoring system ECOTOX in an integrated early-warning system for drinking water**

NETTO, I.<sup>1</sup>, BOSTAN, V.<sup>1</sup>, MCCARTHY, L.<sup>1</sup> and LAURSEN, A.<sup>1</sup>. <sup>1</sup>Ryerson University

Behavioural changes are capable of providing much faster responses than standard 24-h and 48-h bioassays. This study assessed the suitability of the ECOTOX image analysis system, which detects behavioural changes, for inclusion in an integrated early-warning system for drinking water quality to be implemented in Canada. Results of behavioural parameters measured by ECOTOX using the flagellated protist *Euglena gracilis* were compared to visual observations of *E. gracilis* behaviour after exposure to contaminants to determine the automated system's ability to detect behavioural changes. Cells were incubated in a range of concentrations of copper, atrazine, and tributyltin for 1h, 2h, and 24h. Findings indicate that while *E. gracilis* is a very sensitive organism to copper, to which it shows inhibition of motility at low concentrations within 1h, the image analysis system was in general less sensitive than visual observation for detecting behavioural changes. In contrast, after exposure to organic contaminants atrazine and tributyltin, the ECOTOX system detected small changes in the number of cells swimming upwards (antigravitactic behaviour), which indicates that the system has the potential for detecting changes in behaviour which cannot be seen under visual observation.

### **The applicability of standard aquatic testing protocols for nanoscale particles**

KLOSCHINSKY, T.<sup>1</sup>, PETHO, E.<sup>1</sup>, MARQUES, L.<sup>1</sup> and CHAN REMILLARD, S.<sup>1</sup>. <sup>1</sup>Hydroqual Laboratories and Golder Associates Ltd.

The use of nanoscale particles in consumer and industrial products is increasing very rapidly with seemingly endless applications for these particles. However, parallel to the evolution of nanotechnology there needs to be an awareness of the ecological impact of these particles on organisms. A major hurdle that the nanotechnology industry faces is the question of how to measure these effects on the environment. Considering many of the issues that encompass the use of nanoscale particles (low solubility, aggregation etc.), can current government-approved ecotoxicity protocols be used to assess the safety of these particles on the environment? The key objective of this research was to determine the suitability of current Environment Canada methods for evaluating toxicity of nanoscale particles to key indicator organisms. To achieve this we needed to identify ways to adapt Environment Canada methods, within acceptable guidelines, to alter the colloidal suspension characteristics of nanoscale particles with low solubility and to measure how these changes in the solubility profiles would affect toxicity of nanoscale particles to environmental indicator organisms. Test media conditions such as pH and water hardness levels were altered and surface active agents were added to affect colloidal suspension characteristics. The resulting changes in colloidal suspension characteristics and toxicity profiles will be discussed.

### **Further developments in the use of whole-organism responses in the "miners' canary"**

MCCARTHY, L.<sup>1</sup>, LAURSEN, A.<sup>1</sup>, BOSTAN, V.<sup>1</sup>, PUSHCHAK, R.<sup>1</sup>, MEHRVAR, M.<sup>1</sup> and GILBRIDE, K.<sup>1</sup>. <sup>1</sup>Ryerson University

Chemical analyses of potentially stressed aquatic systems are slow, expensive, and limited to the chemicals that standards have been developed for. We have developed a "miners' canary" of relevant aquatic biomonitoring organisms to measure stress reactions in the aquatic community in real-time. Graded ranges and patterns of behaviours have been incorporated into ecotoxicity models based on dose-response of individual organisms to specific classes of stressors. These organisms include *Pseudokirchneriella subcapitata*, *Euglena gracilis*, *Daphnia magna*, *Hyaella azteca*, *Lemna minor*, *Chironomus tentans*, and *Lumbriculus variegatus*. Light and dissecting microscopes, respirometers and O<sub>2</sub>/CO<sub>2</sub> microelectrodes, real-time, online image analysis, and automated biomonitoring systems were all used to assess and record behaviours. While initially proposed for regions that take their drinking water from Great Lakes systems such as the Welland Canal, it is their use in marginalized areas such as First Nations drinking water plants downstream of industrial effluent that may realize its true value. The ability to assess the sustainability of an urban watershed can have enormous potential. For example, the "miners' canary" can assess whether a potentially contaminated wastewater system has been improved and whose effluent will no longer have impact on receiving-water biota. This system is also easily transferred internationally, particularly where the quality of drinking water is suspect, but where monies and know-how for chemical analysis are lacking.



### **In vivo Solid-Phase Microextraction (SPME) for monitoring of pharmaceuticals in fish (PO)**

ZHANG, S. <sup>1</sup>, OAKES, K. <sup>1</sup>, METCALFE, C. <sup>2</sup>, PAWLISZYN, J. <sup>1</sup> and SERVOS, M. <sup>1</sup>. <sup>1</sup>University of Waterloo, <sup>2</sup>Trent University

High spatial resolution is a prerequisite for determining local analyte concentrations within heterogeneous living systems. To ensure spatial resolution and resolve rapid processes such as bioconcentration and metabolism of analytes, temporal resolution must also be considered. Accordingly, space-resolved SPME was developed to monitor the tissue specific bioconcentration of nine PhACs in adipose and dorsal-epaxial muscle tissue in free-moving rainbow trout (*Oncorhynchus mykiss*) during a 14 d lab exposure. In this experiment, a SPME fiber with two segmented medical grade poly(dimethylsiloxane) coatings served as the extraction phase, with one segment in direct contact with adipose tissue, and the other with dorsal-epaxial muscle. The relative bioavailability of PhACs in the two tissues was calculated by comparing the extracted amount of analyte in equilibrium. The dynamic bioconcentration processes of five PhACs (atrazine, gemfibrozil, carbamazepine, ibuprofen, and fluoxetine) were monitored, demonstrating bioconcentration is a tissue-specific process. The remaining four analytes (atorvastatin, naproxen, bisphenol A, and diclofenac) were detected in the fish exposure water, but were not detected in fish tissues, presumably reflecting a lack of bioconcentration potential inherent to their chemical properties.

### **Pulsed exposure toxicity of glyphosate, MON 0818 and two commercial formulations *Daphnia magna*: Recovery capacity after short term exposure (PO)**

RODRIGUEZ-GIL, J. <sup>1</sup>, HANSON, M. <sup>2</sup> and SOLOMON, K. <sup>1</sup>. <sup>1</sup>University of Guelph, <sup>2</sup>University of Manitoba

Previous studies have shown technical glyphosate to be of low toxicity to aquatic organisms. However, most commercial formulations include formulants for increased product efficacy. Many studies have compared the aquatic toxicity of technical glyphosate, commercial formulations, and a number of commonly used formulants, paying special attention to the polyethoxylated tallowamine (POEA) surfactant MON 0818. In general these studies have shown this surfactant to be more toxic than glyphosate alone. Although commercial formulations containing MON 0818 are not intended for over-water uses, its use in forestry can lead to unintended exposure of forest pools and ponds. The pulsed nature of the application of glyphosate-containing products and its relatively short half-life in the water column leads to acute exposures under environmental conditions. In order to improve the accuracy of ecotoxicological risk assessments for these stressors, a greater understanding of the effects that pulsed exposures have on aquatic organisms is needed. Time-to-event analysis of the toxicity of technical glyphosate, the MON 0818 surfactant and two commercial formulations containing MON 0818 to the cladoceran *Daphnia magna* was performed. Test organisms were transferred to fresh media after exposures of 2, 4, 8, 16, 24, 48 and 96 h and reproduction after a 14 days recovery period was assessed. EC50 values were calculated after initial exposure and after recovery. The MON 0108 surfactant and both commercial formulations showed reduced EC50 values relative to glyphosate alone. Short exposures to the concentrations tested allowed for recovery in the tested organisms with hormetic effects observed in post-exposure algal growth



### **The potential use of Atlantic silverside (*Menidia menidia*) as an indicator of pollution in Saint John Harbour, New Brunswick (PO)**

DOYLE, M.<sup>1</sup>, BOSKER, T.<sup>1</sup> and MUNKITTRICK, K.<sup>1</sup>. <sup>1</sup>University of New Brunswick Saint John

Although estuarine and marine environments are commonly impacted by numerous (non) point sources, there is a lack of standard monitoring techniques to assess these impacts. In order to assess the impact of contaminants, we evaluated the use of Atlantic silverside (*Menidia menidia*), as a sentinel species. Silverside were caught along a gradient of pollution during their onshore period from April to November in the Saint John River Estuary, New Brunswick, to determine whether they would be good indicators of pollution. When young of the year fish returned from offshore in the spring there were no differences in length, weight, gonad size or condition factor between sites. During the prespawning phase, a difference in condition factor appeared gradually between sites and increased during the onshore period. Gonad size peaked in early June, prior to the fish disappearance during the spawning season, and no differences in GSI were found among sites prior to spawning. By September, most fish collected were young of the year. At the exposed site, there was a significant bias towards female juveniles relative to the reference site. There was also a graded response in condition factor consistent with levels of contamination. The gradient of differences between sites and the increase over time suggests that Atlantic silverside may be locally resident during their onshore period and therefore represent location conditions. Based on these results, Atlantic silverside have potential to be used as a sentinel species and sampling should focus on an early June period prior to the full moon to ensure maximal exposure periods before spawning, or in late October to evaluate growth as fish grow rapidly prior to offshore movement.

### **Spatial distribution of mercury in breeding common terns along the upper St. Lawrence River (PO)**

BAIRD, C.<sup>1</sup>. <sup>1</sup>Queen's University

Common terns (*Sterna hirundo*) return annually from the Caribbean and South America to breed on the St. Lawrence river along a southwest-northeast gradient between the outflow at Lake Ontario and the Eisenhower Lock system, located approx. 170km downriver at Cornwall, Ontario, and Massena, New York. Both Cornwall and Massena are designated as "Areas of Concern" (AOC) by the International Joint Commission (IJC), due to historic discharges of environmental contaminants including mercury (Hg) and polychlorinated biphenyls (PCBs). Because terns are obligate piscivorous, they are useful bioindicators for monitoring the spatial distribution of contaminants within the St. Lawrence aquatic system. This project will use breast feathers of terns to analyze the spatial distribution of mercury on the breeding grounds, with a particular emphasis on comparing Hg for terns breeding within the Massena AOC, and two colonies upriver away from the AOC. Stable carbon (d13C) and nitrogen (d15N) isotopes will be used to compare trophic position among individuals and colonies and to help elucidate the relationship between trophic position and contaminant uptake. In addition, contaminant loads on the breeding grounds will be compared with those accumulated on the wintering grounds by sampling the same birds twice, first upon return from wintering grounds, then resampling the re-grown feather after the breeding season.

## ***Assessing and Managing Contaminated Sediment***

### **Monitoring the effectiveness of remediation of contaminated sediments using field and laboratory measures of bioaccumulation**

VAN GEEST, J. <sup>1</sup>, BENOIT, N. <sup>2</sup>, FLETCHER, R. <sup>2</sup> and POIRIER, D. <sup>2</sup>. <sup>1</sup>University of Guelph,  
<sup>2</sup>Ontario Ministry of the Environment

Contaminated sediments are a major environmental concern as they may be a source of persistent and toxic compounds that can be mobilized and transferred into and through the food web via the processes of bioaccumulation and biomagnification. An assessment of the potential for the biomagnification of contaminants is now one of the four lines of evidence in the Canada-Ontario Decision Making Framework for Assessment of Great Lakes Contaminated Sediment and can be conservatively modeled as an initial line of evidence. However, further investigation to fully assess the risk associated with bioaccumulation/biomagnification requires tools such as field collections and in-situ or laboratory studies. These biological-based tools are also useful to assess the effectiveness of remediation efforts at contaminated sites. Recently, the Ontario Ministry of the Environment completed development and standardization of a laboratory protocol for measuring bioaccumulation of sediment-associated contaminants in freshwater invertebrates and fish. Two case studies are presented of sites in Ontario that have undergone remediation (removal) of contaminated sediment. The effectiveness of the remediation efforts are discussed based on bioaccumulation data from the field and the new laboratory method.

### **The role of ecological risk assessment in the delisting of the Wheatley Harbour Area of Concern**

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In 1985, 43 Areas of Concern (AOCs) were identified by Canada and the United States pursuant to the Great Lakes Water Quality Agreement. To date, three AOCs in Canada and one AOC in the United States have been delisted, of which Wheatley Harbour, located in Lake Erie, is the most recent. During the delisting process, ecological risk assessment can be a valuable tool to inform the Remedial Action Plan for an AOC. A targeted ecological risk assessment was conducted for the Muddy Creek wetland portion of Wheatley Harbour to determine whether sediment remediation was warranted to mitigate risks from polychlorinated biphenyls (PCBs) to fish and piscivorous wildlife. PCBs in sediment and biota of Muddy Creek are attributed to historical discharge of wastewater and disposal of fish offal by nearby fish processing plants. Multiple lines of evidence were considered in the assessment by varying the basis for both exposure terms and the thresholds employed in the calculation of hazard quotients. To ensure the protectiveness and completeness of the analysis, risk-based management goals for fish tissue and sediment were calculated. The ecological risk assessment concluded that sediment remediation in Muddy Creek was not warranted. Through this determination, the Wheatley Harbour AOC was able to move forward to delisting.

### **Sediment remedial options for Lyons Creek East (Niagara River Area of Concern)**

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Historic PCB contamination of the sediments in Lyons Creek East was identified in the RAP Phase II as one of the sites within the Niagara River Area of Concern that needed further assessment. A risk-based approach was used to determine whether PCB contamination of the sediments posed a risk to human health and biota, and to develop a suitable remedial strategy. The potential ecological risks were found to be the more sensitive, and were the drivers for developing a remediation plan. The potential risks were assessed, and remedial options were considered with respect to the designation of the area as a Provincially Significant Wetland. The approach considered the risks, and in particular the uncertainties upon which ecological risk assessments are based, with respect to the need to protect the wetland and the threatened species known to be present. The remedial options considered the destructive nature of intrusive remedial options and, based on the risks identified, recommended Monitored Natural Recovery as the most appropriate remedial strategy, given the potential risks to biota from exposure to PCBs and the intrinsic value of the habitat.

### **New Canadian framework for addressing and managing aquatic contaminated sites**

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A common risk-based framework for the adaptive management of contaminated aquatic sites under Canadian federal custody is described. The framework is a 10-step process which begins with Problem Formulation (Steps 1-2), during which suspect aquatic sites are identified for further assessment and aquatic sites that are not suspected of being contaminated are eliminated from further consideration. Steps 3-4 involve Screening Level Risk Assessment, during which contaminated aquatic sites are either: identified as requiring risk management (e.g., remediation); classified for further assessment; or, eliminated from further consideration. Steps 5-6 involve Detailed Level Risk Assessment, following which aquatic sites are either prioritized for further management action(s) or eliminated from further consideration. Risk Management strategies are developed and implemented for prioritized contaminated aquatic sites in Steps 7 and 8. Risk Management monitoring (confirmatory sampling and long-term monitoring) is conducted in Steps 9 and 10 to ensure that risk management objectives are met. The framework is iterative and sequential in both scope and decision points (the latter comprise simple "yes" or "no" criteria). It is intended to be sufficiently prescriptive to standardize the decision-making process while still allowing for necessary site-specific flexibility. Aquatic sites entering the process can be eliminated from further consideration at three decision points or can be prioritized for management action(s). Contaminated aquatic sites where management action(s) are necessary remain within the process until successful remediation has been achieved and confirmed. Successful remediation is defined as a condition where there are negligible risks to human health or the environment.

## **Aquatic contaminated site management framework for the Federal Contaminated Sites Action Plan (FCSAP) (PO)**

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The Federal Contaminated Sites Action Plan (FCSAP) provides funding to federal departments, agencies and consolidated Crown corporations in Canada to manage the human health and ecological risks associated with their contaminated sites and to reduce the associated federal financial liability. Contaminated site management in the FCSAP program has historically followed a common 10-step approach. Although it has been an effective management tool for terrestrial contaminated sites, the approach lacked specific guidance on addressing contaminated aquatic sites and sediments. Therefore, a risk-based framework for the adaptive management of contaminated aquatic sites was developed. Following a 10-step approach similar to the one used for terrestrial sites, the aquatic site framework is iterative and sequential in both scope and decision points. The Framework is intended to be sufficiently prescriptive to standardize the decision-making process while still allowing for necessary site-specific flexibility.

## **Uptake and effects of heavy metals from spiked sediments in zebrafish embryos (PO)**

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Predicting the bioavailability of heavy metals in sediments is of major concern within sediment risk assessment. The present study investigated the extent to which heavy metals from spiked sediments are bioavailable and accumulate in zebrafish (*Danio rerio*) embryos as a model for sediment-living organisms. *Danio rerio* embryos were exposed to a natural and an artificial sediment spiked with cadmium, copper, nickel and zinc, individually and as mixture, respectively. Following exposure, the fish embryos were digested and heavy metal concentrations were measured by means of inductively coupled plasma-mass spectrometry (ICP-MS). Additionally, toxicity tests with corresponding metal solutions and direct sediment contact tests were carried out.

Toxicity of metals from sediments to zebrafish embryos was lower by a factor of up to 10<sup>2</sup> compared to aqueous solutions. In the uptake-experiments, *Danio rerio* eggs accumulated heavy metals from spiked sediments in up to a few hundred times higher concentrations compared to the spiked concentration. Copper accumulated significantly higher than the other heavy metals. Due to a higher content of organic matter and higher bacterial activity reducing bioavailability of the tested natural sediment, all heavy metals accumulated to a higher extent after exposure to the artificial sediment. The presence of other heavy metals reduced the uptake of individual metals. Due to the minimal applied equilibration time, the obtained results represent the bioavailability of heavy metals from sediments immediately after the initial contamination event.



## **Ecological Risk Assessment for dredged sediment management in the St. Lawrence River (PO)**

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The St. Lawrence River undergoes various anthropological pressures (e.g., navigation) which can entail negative consequences for the ecosystem. Every year, thousands of cubic meters of sediment are dredged along this waterway to maintain secure navigation. Dredged sediments are, in many cases, deposited in open water. As a result of the 3rd and 4th St. Lawrence Action Plan, the current vision of a sustainable management of this river and its main functions entails the need for sound risk-based assessment approaches to support management decisions. More specifically, the sustainable navigation strategy, drawn up under the St. Lawrence Action Plan III, explicitly identified the need to develop sediment quality assessment tools, including those derived from ecotoxicological studies. The first management option addressed in this perspective was the open water disposal of dredged sediment. In this context, an ecotoxicological risk assessment (ERA) approach using a chemical characterization in Tier 1 and bioassays using benthic organisms in Tier 2 was elaborated on the basis of physicochemical characterization, toxicity testing and benthic community structure determination of sediment samples collected in 59 sites along the St. Lawrence River. This ERA allows estimating if the risk posed by the dredged sediment exposure to benthic organisms, at the deposit site and downstream, is acceptable and compatible with the open water disposal.

## **Mutagenic activity in sediments and fish samples of the quarry pond Karlskopf of River Rhine: Linking effects from the laboratory to the field (PO)**

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Various international studies have revealed a correlation between contamination of sediments and adverse effects on fish biocenosis. A decline in the number and biomass of fish has not only been reported for the River Rhine but also for many other rivers. In the context of the European Water Framework Directive (WFD), the causes for this decline are currently being evaluated. Nonetheless, sediments as an integral factor of water quality and one major source of priority pollutants have not yet been sufficiently considered. To expand the range of biotests of a Sediment Quality Triad (SQT) conducted by Schulze et al. on the quarry pond Karlskopf, a backwater of the River Rhine, genotoxicity and mutagenicity were assessed. The Micronucleus Test with cardiac erythrocytes of *Abramis brama* and *Rutilus rutilus* as biomarker for in situ genotoxicity and the Ames Fluctuation Assay with acetonitrile extracts of sediments from three different depths were performed. No significant (or only minor) genotoxic or mutagenic effects were found in the experiments, both in vitro and in situ, elucidating that genotoxicity seems to be of minor relevance in this quarry pond. Overall, both tests conducted show similar results in the observed range. However, the fish sampling



procedure should be modified. Electric fishing is a more suitable method because injuries to the fish by gillnets is prevented. For the completion of the SQT, the structure of fish and macrozoobenthos biocoenosis, as well as histopathology of fish, could be investigated in future. The RWTH Aachen University Undergraduate Funds, as part of the German Excellence Initiative, provided funding for participation in the ATW in the form of a personal travel grant to the first author.

#### **Butyltins in sediments of the St. Lawrence River and Montreal Harbour (PO)**

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Butyltins have been used as biocides in antifouling paints for ship hulls, in fungicides, insecticides and wood preservation products and as stabilizers in polyvinyl chloride (PVC) since 1960. Although their use on small vessels has been regulated in Canada since 1989, butyltins are still used as an antifouling agent by a significant portion of the worldwide commercial fleet. Butyltins can be found in sediments in the form of tributyltin and its degradation products, dibutyltin and monobutyltin. These organic metals are persistent in the environment, bioaccumulate in aquatic organisms and cause toxicity. As part of the collaboration between monitoring and research programs on chemical contamination in the St. Lawrence River (Canada), we analyzed butyltins in 250 sediment samples carry out in fluvial lakes, commercial harbours, and marinas along the river. Elevated concentrations of butyltins, as high as 1400 ng Sn g<sup>-1</sup>, were found in sediments of the Montreal harbour. Concentrations up to 200 ng Sn g<sup>-1</sup> were also measured in some marinas. Much lower concentrations (< 50 ng Sn g<sup>-1</sup>) were reported in the fluvial lakes away from the main harbour zones. Contamination by butyltins was also found in the river section between Montreal and Sorel, particularly in the vicinity of the Iles-de-Contrecoeur National Wildlife Reserve, where an extremely high concentration of 2100 ng Sn g<sup>-1</sup> was measured in surface sediments. Tributyltin accounted for a high percentage of total butyltins levels (28-60%), indicating that contamination by butyltins still represents a source of potential toxicity for the St. Lawrence River ecosystem.

### ***"Omics" in Toxicology***

#### **Molecular toxicity identification evaluation in *D. magna***

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Current methods to identify the underlying cause of toxicity, or toxicology identification evaluation (TIE), are limited in their ability to rapidly, specifically, and cost-effectively identify toxic chemicals in an effluent. We describe our development of molecular TIE (mTIE) approaches that use gene expression to identify the contaminant(s) to which an organism has been exposed. *Daphnia magna* is ideal for this work because of its small size, easy culture,

rapid generation time, and sensitivity to a diversity of contaminants. We have carried out gene expression profiling to 36 EPA priority pollutants at equitoxic concentrations using a new 15K *D.magna*, Agilent array developed by an international consortium of *D. magna* researchers. Each contaminant produces a distinct expression profile, and we identified 2738 genes which are differentially expressed in at least one exposure. We will describe our utilization of computational approaches to robustly discriminate between each contaminant. For example, we utilized the Hierarchical Ordered Partitioning and Collapsing Hybrid (HOPACH) algorithm which is a hybrid of divisive and agglomerative methods with bootstrapping to assess cluster assignment as one approach. We are also investigating various classifier approaches including random forest for development of robust discriminators between exposures. We will also present the development of predictive models based on advanced machine learning methods which aims to identify predictive signatures based on the synergistic effects of small gene subsets. These methods have been already validated on a number of other systems and proved to have the potential to provide accurate mechanism-based biomarkers.

#### **Omics and cell signaling cascades in the teleost brain: Implications for pesticide exposures and neurodegeneration?**

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Environmental exposures to the pesticide dieldrin have been linked to human neurodegenerative diseases. Although the use of dieldrin is restricted, it continues to be a problem in some agricultural areas. The primary mechanism of dieldrin is to block GABA-A receptors in the CNS resulting in mortality. To better understand the mechanisms of dieldrin action in the fish brain, three independent injection and feeding experiments were performed using largemouth bass, a species that accumulates high levels of dieldrin. Microarray data suggest that there is a significant genomic response of the ubiquitin-proteasome pathway to dieldrin that is associated with a change in GABA levels within the brain. In addition, the biological processes of stress, DNA repair, and signalling cascades are significantly affected by dieldrin. Thus, transcriptome data suggests protein degradation pathways and DNA repair mechanisms are altered after dieldrin exposure. Proteomic responses as determined by iTRAQ methodology identified proteins that are significantly altered in abundance by dieldrin. These included apolipoprotein E, microtubule associated Tau protein, and enolase A. Most interesting is that these proteins are also associated with neurodegenerative diseases in humans and are used as neurological biomarkers. Lastly, there appears to be sexually dimorphic responses to dieldrin at the genomic level. This may be important because human males are more frequently afflicted with neurodegenerative diseases than females. Therefore, males and females respond differently to some environmental contaminant exposures. The implications for understanding gene-protein relationships and the power of bioinformatics approaches to better understand mechanism of action in aquatic toxicology will be discussed.

## **Ionic silver and nanosilver as novel endocrine disruptors in frogs at environmentally relevant, low concentrations**

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Nanosilver is the most commonly used nanometal in consumer products. It has strong antibacterial properties and has been incorporated into many consumer products. Nanosilver has a high likelihood of contaminating aquatic systems as a constituent in the effluent from municipal and industrial effluent discharge and entering cells of aquatic organisms, yet little is known regarding its biological effects. The present study evaluated the toxicological effect of nanosilver and ionic silver at concentrations around current water quality guidelines for the protection of aquatic organisms. Frog postembryonic development is a process that is particularly sensitive to aquatic contaminants. They undergo a thyroid hormone (TH)-dependent metamorphosis from tadpole to juvenile frog whose perturbation can serve as an indicator of disruption of TH action. We exposed premetamorphic *Rana catesbeiana* (American bullfrog) tadpoles to 0.06 – 6.0 µg L<sup>-1</sup> nanosilver or ionic silver for up to 28 days and found that both forms of silver alter metamorphosis in distinctive ways. In order to elucidate pathways affected by silver exposure, we examined multiple tissues using combined transcriptomics (microarray/QPCR; quantitative real time polymerase chain reaction), proteomics (iTRAQ; isobaric tags for relative and absolute quantitation), and metabolomics (DI-FTICR; direct infusion-Fourier transform ion cyclotron resonance) techniques. Our results reveal some surprising insights into the mode of action of different forms of this chemical. Moreover, the levels of silver that elicited an effect on this important environmental sentinel species were at or below North American water quality guideline levels for silver, suggesting that a re-evaluation of these guidelines is warranted.

## **DNA microarray-based analysis of gene expression in livers of fathead minnows (*Pimephales promelas*) exposed to pulp and paper mill effluents (PO)**

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Pulp and paper mill effluents have been linked to adverse effects in fish populations present in mill receiving environments that are characteristic of endocrine disruption. These include impaired reproduction, development and survival. While the physiological effects are well characterized, the molecular mechanisms behind these effects are not yet understood. In order to investigate the transcriptional effects induced by exposure to different pulp and paper effluents, male and female fathead minnows were exposed for 6 days to 10% untreated kraft effluent (UTK), 25% secondary treated kraft effluent (TK) or 100% combined mill outfall (CMO) from a combined kraft pulp and newsprint mill. Gene expression changes in the liver were analyzed using a 22K oligonucleotide microarray. Exposure to UTK, TK and CMO resulted in significant changes in the expression levels of 118, 110 and 182 targets, respectively, in male fish and 187, 454 and 298 targets, respectively, in female fish. The data provide an overview of different pathways affected before and after secondary treatment and show the existence of clusters of genes that are up/down regulated in all treatments as well as genes that are differentially regulated according to treatment. Functional analysis using gene

ontology identified sterol, isoprenoid, steroid, and cholesterol biosynthesis as biological processes altered by exposure to pulp and paper mill effluents.

## ***2-day Posters***

### **Everything you wanted to know about Laurentian SETAC but were afraid to ask (PO)**

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Laurentian SETAC is the Ontario chapter of the Society of Environmental Toxicology and Chemistry (SETAC). We have about 150 members from academia, government and consulting/industry. Students make up a third of our membership. Our mission is to promote and enhance professionalism, communication, and education in environmental toxicology and chemistry. We are a dynamic organization with an active membership. Each year we hold an Annual General Meeting and Conference in June—an exciting day of plenary presentations, posters, platform presentations, social events and student awards. We also have a series of pub nights between September and May in both Ottawa and southern Ontario, where members attend an in-depth scientific presentation over dinner in a casual atmosphere of socializing and networking. Short courses are also offered (typically once every year) to provide professional development opportunities. Recently offered short courses include "Ecotoxicological Risk Assessment" and "Radioactivity in the Environment". We are also active in the community by participating in science fairs and planting trees to offset our carbon footprint associated with our annual meeting. Students are supported by reduced rates for membership and to attend events, awards for best presentations at our general meeting, and through job postings/career fairs. Everyone is welcome to join and take part in our activities. To find out more about Laurentian SETAC, please visit our website at [www.laurentiansetac.ca](http://www.laurentiansetac.ca)

### **"IGETG" it . . . do you? (PO)**

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Toxicity testing is an integral component of environmental monitoring and compliance, environmental assessments and guideline development. To date, 29 standardized test methods and supporting guidance documents have been published by Environment Canada, which reflects the contributions of researchers, data-generating laboratories, regulators and users of the data. Efforts to make these methods consistent and less variable across Canada have been facilitated by the actions of an ad-hoc group, the Inter-governmental Ecotoxicology Testing Group (IGETG). IGETG serves as a mechanism by which both federal and provincial government agencies in Canada work together to promote a consistent approach to testing with internationally recognized quality standards. The IGETG has five objectives: 1) developing, validating and publishing new toxicological test methods; 2) promoting the use of toxicity testing in regulations and policies; 3) dissemination of new knowledge and understanding the issues related to ecotoxicology testing; 4) providing scientific support to environmental conservation and protection programs; and 5) working to establish and implement quality assurance practices in Canadian toxicology laboratories. Areas of research

in which IGETG members are playing an active role include the development of microscale tests, reducing the number and types of organisms sacrificed in toxicity testing; the use of microarrays for both rainbow trout and amphibians; harmonizing the federal and Ontario provincial sediment testing procedures; developing a bioaccumulation test method for estimating uptake from sediment, food and/or water; and participating in the study of recent declines in amphibian populations in close collaboration with Canadian Wildlife Service scientists. This poster serves to inform those unaware of IGETG and its membership of the programs we support and our current activities.



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